

ORIGINAL PAPER

Development of a chitosan-based facial mask incorporated with *Caesalpinia sappan* heartwood crude extract

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Abstract. Crude extract from *Caesalpinia sappan* heartwood incorporated into a chitosan-based facial mask has potential applications in inhibiting *Staphylococcus aureus* causing infections on dermatitis injuries. This study aimed to evaluate the antibacterial activity of *Caesalpinia sappan* heartwood crude extract against *S. aureus* and to develop a chitosan-based facial mask incorporating the extract for acne-related inflammation caused by this bacterium. The heartwood was extracted using 95% ethanol and its antibacterial activity was evaluated using the disc diffusion method, with 1,000 ppm clindamycin as a positive control. The crude extract at concentrations of 40,000 ppm, 4,000 ppm, and 400 ppm, along with clindamycin, demonstrated clear inhibitory effects against *S. aureus*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were 156.25 ppm and 20,000 ppm, respectively. Subsequently, a chitosan-based facial mask formulation containing 20,000 ppm of the extract was developed and evaluated. The results showed that the developed mask exhibited effective antibacterial activity against *S. aureus* while maintaining desirable physical properties, indicating its strong potential for use as an antibacterial facial treatment.

Keywords: *Caesalpinia sappan*, *Staphylococcus aureus*, Disc diffusion method, Chitosan-based facial mask

1. Introduction

The inflammation of facial is mostly caused by malfunctioning of lipid layer, associated with histamine released from mast cell (Gschwandtner et al., 2012). Another cause is *Staphylococcus aureus* infections which can be treated by antibiotics. Even though using drugs can treat the bacteria rapidly, it can lead to drug residue and bacterial drug resistance. Plant-based bioactive compounds and crude extracts with strong antimicrobial properties

are considered as sustainable, eco-friendly, and cost-effective alternatives in medicine. Many biological activities of sappan heartwood (*C. sappan* L.) have been traditionally used as a natural coloring agent in beverage, food, garment, medicine and cosmetic. It has been reported that antimicrobial activities and inhibitors of micro-pathogen. *Caesalpinia sappan* heartwood containing brazillin exhibits antibacterial properties against several gram-positive bacteria, such as *Bacillus subtilis*, *S. aureus* and gram-negative bacteria, such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus vulgaris* (Piyaboon, 2025). Therefore, using *C. sappan* heartwood crude extract to treat inflammation is potentially viable method since *C. sappan* heartwood consists of brazillin, one of the flavonoids which can reduces MRSA (Methicillin-resistant *S. aureus*) virulence by decreasing secretion of α -hemolysin (Hla) via inhibition of the response regulator *SaeR* of the *SaeRS* two-component regulatory system of *S. aureus* (Li et al., 2025). The market demand for facial masks is growing in the cosmetic industry. Application of facial masks provides targeted dermatological benefits, including improved skin hydration, anti-wrinkle action, anti-inflammatory support and cellular rejuvenation. Nowadays, facial masks have undergone modifications, with new aspects, ingredients, and vehicles offering new perspectives for skincare. In recent years, hydrogel-based facial masks have been widely investigated as a promising biomaterial, which are non-toxic, photocurable materials, exhibit significant potential for use in facial masks.

Chitosan is a nontoxic, biodegradable and biocompatible polysaccharide derived from partially deacetylated chitin. Chitosan has many free amino groups and carries a positive charge that interact with negative charges on the surface of epithelial cells and increases the permeability of the epithelial membrane. In addition, Chitosan is widely known for its film-forming ability and computability with natural plant extracts to form an adhesive film and more improved adhesion, controlled release of active compounds, prolonged skin residence time, or enhanced stability (Lia et al., 2018).

The objectives of this research were to ability to inhibitory activity against *S. aureus* of *C. sappan* heartwood crude extract and development of facial mask from *C. sappan* heartwood crude extract and chitosan.

2. Methodology

2.1 *Caesalpinia sappan* heartwood crude extract preparation

Dry powder of 1,200 g *C. sappan* heartwood were fully dried using hot air oven at 65°C for 72 h. Then use a dry weight of *C. sappan* heartwood powder to extract by 95% ethyl alcohol in the ratio of powder to ethyl alcohol was 1 g: 5 mL. The sample solutions were incubated at 30°C and shaken at 150 rpm for 5 days after solvent evaporation by rotary evaporator at 50°C. The *C. sappan* heartwood crude extract of 67.68 g, calculated as percent yield of 5.64%. The extract was stored in sealed containers and refrigerated at 2-4 °C.

2.2 Evaluation of antibacterial activity in vitro

Staphylococcus aureus was cultured using nutrient agar (NA, Hi-media) at 37°C for 24 h. Then it was diluted to concentration of 10⁸ CFU/mL, 0.5 McFarland's standard, 100 mL and spread on NA in petri dishes. *Caesalpinia sappan* heartwood crude extract was prepared by diluting using 1% DMSO into 400 ppm, 4,000 ppm and 40,000 ppm concentration.

Negative control was 1% DMSO which solvent control and positive control was 1,000 ppm of clindamycin antibiotic following concentration standard inhibitory concentration bacteria. Then for antimicrobial testing, 30 microliters of crude extract in each concentration were dropped on paper disc (6 millimeters diameter) and placed on prepared 10⁸ CFU/mL of *S. aureus* on NA and incubated at 37°C for 24 h. The antimicrobial tests were repeated for three times. Measure the inhibition zone (clear zone). The obtained values were used to calculate the mean and standard deviation, which were then compared for antibacterial efficacy using a one-way ANOVA. Significant differences among the treatments were determined using Duncan's Multiple Range Test (DMRT).

Determination of minimum inhibitory concentration (MIC)

The resazurin-based turbidometric (TB) method was used to study the antimicrobial efficacy of *C. sappan* heartwood extract against *S. aureus* (O'Brien et al., 2000). Serial two-fold dilutions of *C. sappan* heartwood extract in concentrations ranging from 78.12 ppm to 40,000 ppm with adjusted final inoculum of bacterial concentration (10⁸ CFU/mL) were used to determine MIC in NB. The positive control contained 1,000 ppm of clindamycin antibiotic and the negative control contained 1% DMSO incubated at 37°C for 24 h. After incubating, filled 0.15 mg/mL of resazurin solution used (50 mL per well) and incubated again at 37°C for 24 h. Observe the changes of resazurin. A change in color from blue (oxidized state) to pink (reduced state) indicated growth of the bacteria. The lowest concentration prior to color change was considered as the MIC.

Determination of the minimum bactericidal concentration (MBC)

The minimum biocidal concentration (MBC) was determined from result of the MIC of *C. sappan* heartwood extract which determination showed no visible bacterial growth. MIC test were prepared in 10 µl volumes and dropped onto nutrient agar (NA) plates. These plates

were incubated at 37°C for 24 h. The result was observed pre-incubated and post-incubated agar plates for the presence or absence of bacteria. MBC was defined as the lowest concentration of the test compound to kill the bacteria.

2.3 Facial mask forming using *C. sappan* heartwood extract and chitosan

Two point five grams of chitosan powder was dissolved in 42.5 mL of distilled water with 5 mL of 1% acetic and mixed by magnetic stirrer at 40°C for 40 min. Then add 1 mL of glycerol and continue stirring for 20 min. The chitosan solution was added *C. sappan* heartwood extract at MBC concentration (the lowest concentration to kill the bacteria) into chitosan solution and mixed with magnetic stirrer at 40°C for 40 min. 1% Tripolyphosphate was slowly added while the solution was being stirred. This protocol was similarly used for positive (1,000 ppm of clindamycin) and negative (1% DMSO) chitosan film forming. After stirring, poured the solution onto the facial mask mold and let dry at 25°C for 3 days.

2.4 Facial mask antimicrobial activity

The antibacterial activity was observed referred to Salayová *et al.*, 2021 with modification. Cultures were inoculated with *S. aureus* bacteria and incubated overnight at 37°C to adjust turbidity to a McFarland standard of 0.5 and final inoculum levels of 10⁸ CFU/mL. Six millimeters diameter of facial mask from 3 formulas (negative control, positive control and testing) were placed on surface of prepared NA in petri dishes and incubated at 37°C for 24 h. The zones of inhibition formed around the well demonstrated the ability of samples to inhibit the bacteria. The obtained values were used to calculate the mean and standard deviation, which were then compared for antibacterial efficacy using a one-way ANOVA. Significant differences among the

treatments were determined using Duncan's multiple range test (DMRT).

2.5 Facial mask physical properties test

Thickness

Thickness of facial mask was measured by using vernier caliper for 3 times, then the obtained value was calculated in to mean value.

Tensile strength and elongation test

Caesalpinia sappan heartwood crude extract and chitosan facial mask was cut into designed die and specimen (length of 115 mm and a width of 25 mm) and the specimen was clipped to a universal tensile test using a universal testing machine (UTM, model 10ST, Tinius Olsen). Tensile strength was calculated as follow:

$$\text{Tensile Strength (MPa)} = \text{Force (N)} / \text{Area (mm}^2\text{)}$$

Elongation is measured by the equation:

$$\text{Elongation (\%)} = \frac{\text{Length of specimen increase (mm)}}{\text{Initial specimen length (mm)}}$$

Statistical analysis of the data was conducted using ANOVA one-way test and Duncan values were determined at 0.05 levels.

3. Results and Discussion

3.1 Antimicrobial activity

Caesalpinia sappan heartwood crude extracts at different concentrations, including 400 ppm, 4,000 ppm, and 40,000 ppm were demonstrated inhibitory activity against *S. aureus*, resulting in average diameter of inhibition zone of 32.98 mm, 17.93 mm and 7.71 mm respectively. The differences of inhibition zone were statistically significant at a 95% confidence level, as shown in Table 1.

Table 1 The inhibition zone of *C. sappan* heartwood extract at different concentrations for the inhibiting *S. aureus*

Substances	The average diameter of inhibition zone (Mean $\pm$ Standard Deviation) (mm)
40,000 ppm <i>C. sappan</i> heartwood extract	32.98 $\pm$ 2.72 ^{b*}
4,000 ppm <i>C. sappan</i> heartwood extract	17.93 $\pm$ 3.81 ^c
400 ppm <i>C. sappan</i> heartwood extract	7.71 $\pm$ 2.01 ^d
1,000 ppm Clindamycin	66.25 $\pm$ 5.74 ^a
1% DMSO	0.00 $\pm$ 0.00 ^e

* Means $\pm$ SD in the same column followed by a common letter were not significantly different by DMRT ($p < 0.05$).

Table 2 Minimal inhibitory concentration (MIC) and minimal bacterial concentration (MBC) obtained for *C. sappan* heartwood on *S. aureus*

<i>Caesalpinia sappan</i> heartwood crude extract concentration (ppm)	Inhibitory effects	
	MIC	MBC
40,000.00	+	+
20,000.00	+	+
10,000.00	+	-
5,000.00	+	-
2,500.00	+	-
1,250.00	+	-
625.00	+	-
312.50	+	-
156.25	+	-
78.125	-	-

+ indicates positive antibacterial activity; - indicates no antibacterial activity.

The crude extract from *C. sappan* heartwood at a concentration of 40,000 ppm was initial concentration used for determining the minimum inhibitory concentration. The results showed that MIC of the crude extract against *S. aureus* was 156.25 ppm while MBC was 20,000 ppm, as presented as single values representing the lowest concentrations that inhibit bacterial growth and exhibit bactericidal activity, respectively.

3.2 Characterization of facial mask forming using *C. sappan* heartwood extract and chitosan

Characteristics of facial mask was aimed to know the percentage of each component contained in *C. sappan* heartwood extract and chitosan content so that known the quality of facial mask produced. The result of characteristic was provided inside the Figure 1 as follows.

3.3 Facial mask antimicrobial activity

Three types of facial mask were tested as follows: 1) facial mask from chitosan and *C. sappan* heartwood crude extract, 2) facial mask from chitosan and clindamycin 1,000 ppm (positive control) and 3) facial mask from pure chitosan (negative control). The results showed inhibition zone diameter was 17.20 mm, 47.21 mm and 4.25 mm respectively. The differences of all inhibition zone were statistically significant at a 95% confidence level, as shown in Table 3.

3.4 Facial mask physical properties

Thickness

Thickness is an important parameter that influences the use of facial mask in the chemical permeability. The thickness of the facial masks ranged from 0.11 to 0.12 mm. Statistical analysis showed that the facial mask thickness was not significant in Table 4.

Tensile strength and elongation

The tensile strength of facial masks was shown in Table 4. The measured tensile strength was 1.32 MPa, 0.84 MPa, and 2.52 MPa of *C. sappan* heartwood extract mask, clindamycin mask and pure chitosan mask, respectively. There were not significant differences among *C. sappan* heartwood extract mask and pure chitosan mask.

Elongation at break

Table 4 showed that the elongation at break contradicted the data on tensile strength of variable types of facial mask. Elongation at break of 3 type of facial mask ranges from 10.65% to 22.27%. Data of elongation at break were not significant differences among *C. sappan* heartwood extract mask and pure chitosan mask.



Figure 1 Facial mask forming by *C. sappan* heartwood extract and chitosan

Table 3 The inhibition zone of facial mask in different formulas for the inhibiting *S. aureus*

Types of facial mask	The average diameter of inhibition zone (Mean $\pm$ Standard Deviation) (mm)
<i>Caesalpinia sappan</i> heartwood extract mask	17.20 $\pm$ 3.50 ^{b*}
Clindamycin mask	47.21 $\pm$ 5.26 ^a
Pure chitosan mask	4.25 $\pm$ 1.75 ^c

* Means $\pm$ SD in the same column followed by a common letter were not significantly different by DMRT ($p < 0.05$).

Table 4 Thickness, tensile strength and elongation of types of facial mask

Types of facial mask	Physical properties (Mean $\pm$ Standard Deviation)		
	Thickness (mm)	Tensile strength (MPa)	Elongation (%)
<i>Caesalpinia sappan</i> heartwood extract mask	0.11 $\pm$ 0.13 ^{a*}	1.32 $\pm$ 0.39 ^{ab}	15.58 $\pm$ 1.38 ^{ab}
Clindamycin mask	0.11 $\pm$ 0.16 ^a	0.84 $\pm$ 0.19 ^b	22.27 $\pm$ 5.02 ^b
Pure chitosan mask	0.12 $\pm$ 0.15 ^a	2.52 $\pm$ 1.17 ^a	10.65 $\pm$ 4.55 ^a

*Means $\pm$ SD in the same column followed by a common letter were not significantly different by DMRT ($p < 0.05$).

4. Discussion

Crude extract from the heartwood by 95% ethanol of the *C. sappan* can inhibit *S. aureus* because the ethanol used in the extraction of brazilin, homoisoflavoid and antimicrobial chemical compounds. Brazilin had antibacterial activity against *S. aureus* because brazilin showed inhibiting cell wall, cell membrane, DNA and protein synthesis of bacteria. This research supports the investigations indicated that brazilin from *C. sappan* heartwood has been showed antibacterial activities including *S. aureus*. The bactericidal effect of brazilin could be inhibited DNA and protein synthesis in cells (Nirmal and Panichayupakaranant, 2015). Moreover, the previous reports by Pattananandecha et al. (2022) indicated that brazilin showed strong inhibitory activity against bacteria because brazilin showed inhibiting cell wall and cell membrane. The crude extract of the *C. sappan* had MIC and MBC values as 156.25 ppm and

20,000 ppm against *S. aureus*., resulting in an MBC/MIC ratio of 128. According to established microbiological standards, an MBC/MIC ratio greater than 4 indicates predominantly bacteriostatic activity rather than a bactericidal effect (Ishak et al., 2024). Because the crude extract, the active antimicrobial compounds were diluted by other non-active plant matrix components. The specific active phytochemicals of *C. sappan* were isolated and purified to effect both the MIC and MBC decrease, which might shift that ratio (Panichayupakaranant et al., 2014).

Facial mask from chitosan and *C. sappan* heartwood crude extract, facial mask from chitosan and 1,000 ppm clindamycin and facial mask from pure chitosan only can form mask and inhibit *S. aureus*. Chitosan is one of component contained the forming quality of facial mask produced (Siri and Wattanutchariya, 2019). Chitosan, a natural biopolymer derived from chitin, has been extensively studied for its compatibility with various natural extracts,

including *C. sappan* heartwood extract, due to its film-forming ability, biodegradability, and inherent antimicrobial properties. The combination of chitosan with *C. sappan* heartwood extract has shown promising results in enhancing the functional properties of composite materials, particularly in applications like food packaging and cosmetics. Incorporating *C. sappan* heartwood extract into chitosan-based films has been shown to significantly improve their antimicrobial properties. A study focusing on chitosan/polyvinyl alcohol (PVA) bio-composite films loaded with *C. sappan* extract demonstrated enhanced antimicrobial activity against resistant bacterial strains, making them suitable for food packaging applications (Lei et al., 2021).

The resulting thickness, tensile strength and elongation at break of facial mask from chitosan and *C. sappan* heartwood extract was not significantly different compared to the control biofilm facial mask from pure chitosan only. Based on the result, it shows that thickness can also affect the tensile strength and elongation (Putra et al., 2019). *Caesalpinia sappan* heartwood extract in facial mask was not effort thickness, tensile strength and elongation at break. This is consistent with the confirmed by Varghese et al. (2023) that the strength of the modified films with 5% and 10% basil leaf extract showed similar tensile strength to that of the control PVA/starch film. Facial mask from chitosan and *C. sappan* heartwood extraction at 20,000 ppm has an ideal physical property for using in cosmetics and skin care products and can inhibit *S. aureus*. A facial mask formulated from chitosan and *C. sappan* heartwood crude extract shows potential as a next-generation advanced facial mask with reduce of inflammation of skin in the future.

5. Suggestions

Future studies should evaluate the antibacterial activity of the chitosan-*C. sappan* heartwood facial mask against other acne-related microorganisms such as *Cutibacterium acnes* and *S. epidermidis*. Additionally, stability testing under various storage conditions is recommended to determine the shelf life of

both the crude extract and the formulated mask. Safety assessments, including patch tests and *in vitro* cytotoxicity assays, should be performed to ensure suitability for human skin, particularly sensitive types. The formulation could also be enhanced by incorporating other natural antibacterial or anti-inflammatory agents, while consumer sensory evaluations would provide valuable feedback on texture, adhesion, and comfort during use. Finally, the chitosan-*C. sappan* heartwood composite film may be further developed for applications in other cosmetic or wound-care products where antibacterial properties are desirable.

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References

- Al-Fadhalah KJ, Aleem RM, Thomas N (2021) Microstructure and texture development in thermomechanically processed leaded brass. *Metals*, 11: 998
- Atsumi H, Imai H, Li S, Kondoh K, Kousaka Y, Kojima A (2011) High-strength, lead-free machinable α - β duplex phase brass Cu-40Zn-Cr-Fe-Sn-Bi alloys. *Materials Science and Engineering A*, 529: 275-281
- Berne C, Andrieu E, Reby J, Sobrino JM, Blanc C (2015) The electrochemical behavior of α , β' -brass in basic NaNO₃ solutions. *Journal of The Electrochemical Society*, 162: C648
- Choucrist J, Zanutto F, Grassi V, Balbo A, Ebn Touhami M, Mansouri I, Monticelli C (2019) Corrosion behavior of different brass alloys for drinking water distribution systems. *Metals*, 9: 649
- Haubner R, Strobl S, Linhardt P (2020) Effects on microstructure and corrosion behavior of a heat-treated

- CuZn36Pb2 brass. Defect and Diffusion Forum, 405: 333–338
- Hoshi Y, Otake Y, Uchisawa Y, Watanabe H, Shitanda I, Itagaki M (2023) Real-time imaging of brass cross-section with dezincification corrosion by electrochemical measurement combined with video observation. Materials Transactions, 64: 885–888
- Jennane J, Ebn Touhami M, Zehra S, Baymou Y, Kim SH, Chung IM, Lgaz H (2019) Influence of sodium gluconate and cetyltrimethylammonium bromide on the corrosion behavior of duplex (α - β) brass in sulfuric acid solution. Materials Chemistry and Physics, 227: 200–210
- Karpagavalli R, Balasubramaniam R (2007) Development of novel brasses to resist dezincification. Corrosion Science, 49: 963–979
- Langenegger EE, Robinson FP (1969) A study of the mechanism of dezincification of brasses. Corrosion, 25: 59–66
- Liu Y, Shang W, Liu H, Huang Z (2012) Effect of annealing temperature on corrosion resistance of cold-rolled strip of complex brass. Applied Mechanics and Materials, 184–185: 959–962
- Loto CA, Loto RT (2018) Corrosion behaviour of heat-treated duplex ($\alpha\beta$) brass in nitric acid. Silicon, 10: 2877–2888
- Mapelli C, Venturini R (2006) Dependence of the mechanical properties of an α/β brass on the microstructural features induced by hot extrusion. Scripta Materialia, 54: 1169–1173
- Michel JH, Powell C, Richardson I, Phull B (2017) Development of copper alloys for seawater service from traditional application to state-of-the-art engineering. Corrosion, C2017-09382: 1–11
- Morales J, Fernandez GT, Esparza P, Gonzalez S, Salvarezza RC, Arvia AJ (1995) A comparative study on the passivation and localized corrosion of α , β , and $\alpha + \beta$ brass in borate buffer solutions containing sodium chloride—I. Electrochemical data. Corrosion Science, 37: 211–229
- Moriarty M, Wu Y, Murray T, Hutchinson C (2021) The effect of phase fraction, size and shape on the dezincification of duplex brasses. Corrosion Science, 184: 109366
- Özhan Doğan S, Ekici B (2020) Effect of hot forging pressure and heat treatment on CW625N lead-free brass alloy dezincification resistance. Acta Physica Polonica A, 137: 545–550
- Pelto-Huikko A, Salonen N, Latva M (2025) Dezincification of faucets with different brass alloys. Engineering Failure Analysis, 169: 109202
- Porter D, Easterling K, Sherif M (2021) Phase transformations in metals and alloys. CRC Press, Boca Raton
- Rivera-Grau LM, Casales M, Regla I, Ortega-Toledo DM, Ascencio-Gutierrez JA, Porcayo-Calderon J, Martinez-Gomez L (2013) Effect of organic corrosion inhibitors on the corrosion performance of 1018 carbon steel in 3% NaCl solution. International Journal of Electrochemical Science, 8: 2491–2503
- Sarver E, Edwards M, Zhang Y (2011) Revisiting dezincification performance testing for brass plumbing devices. Materials Performance, 50: 70–75
- Selvaraj S, Ponmariappan S, Natesan M, Palaniswamy N (2003) Dezincification of brass and its control: an overview. Corrosion Reviews, 21: 41–74
- Toufatzis AI, Pantazopoulos GA, David CN, Sagris DS, Paipetis AS (2018) Final heat treatment as a possible solution for the improvement of machinability of Pb-free brass alloys. Metals, 8: 575
- Toufatzis AI, Pantazopoulos GA, Paipetis AS (2016) Microstructure and properties of lead-free brasses using post-processing heat treatment cycles. Materials Science and Technology, 32: 1771–1781
- Zhou P, Hutchison MJ, Erning JW, Scully JR, Ogle K (2017) An in situ kinetic study of brass dezincification and corrosion. Electrochimica Acta, 229: 141–154
- Zhou Y, Mahmood S, Engelberg DL (2021) Brass dezincification with a bipolar electrochemistry technique. Surface and Interfaces, 22: 100865