*Open Veterinary Journal*, (2024), Vol. 14(4): 973-979
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)

*Original Research*
DOI: *10.5455/OVJ.2024.v14.i4.4*

**Submitted:** 17/10/2023 **Accepted:** 05/03/2024 **Published:** 30/04/2024

# Pharmaceutical activity of sappan wood extract (*Caesalpinia sappan* L.) for treating *Escherichia coli* infection in piglets

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**Abstract**

**Background:** *Escherichia coli* infection is one of the major diarrheal diseases resulting in the loss of pigs at a young age.

**Aim:** This research investigated the antimicrobial activity of *Caesalpinia sappan* wood extract against *E. coli* infection as an antibiotic replacement.

**Methods:** *E. coli* was cultured from diarrheal piglets *and* then used to find the minimal inhibition concentration (MIC). *Caesalpinia sappan* wood extract (500 mg/kg) was used for the treatment of diarrheal piglets compared to antibiotics (enrofloxacin 5 mg/kg) by oral administration. Another three groups of diarrheal piglets were used supplemented feed with 1% and 2% extract compared with commercial feed. Subsequently, *E. coli* enumeration, fecal shape, fecal color, and growth rate were recorded from day 1 to 7.

**Results:** Based on the results, *C. sappan* wood extract could inhibit *E. coli* growth at a MIC of 16–34 mg/ml. The number of colonies did not significantly differ between *C. sappan* wood extract and enrofloxacin treatment groups. A supplemented feed with 1% and 2% *C. sappan* wood extract could improve the fecal shape and fecal score compared to the control group, albeit only in suckling pigs. There were significant differences from the control group on days 4, 5, 6, and 7 ($p < 0.05$). However, the average daily gain did not significantly differ among the three groups.

**Conclusion:** The results indicate that *C. sappan* wood extract could improve diarrheal signs in suckling pigs and can be used as a replacement for antibiotics for organic pig production.

**Keywords:** *Escherichia coli*, Pharmaceutical activity, Piglets, Sappan wood extract, Treatment.

## Introduction

*Escherichia coli* infection, or colibacillosis, in suckling and weaned pigs is one of the major diarrheal diseases in the swine industry, occurring both chronically and sporadically (Castro *et al*., 2022). It may result in the loss of pigs at a young age during the weaning period. This disease is a financial burden in pig production due to the high mortality rate, retarded growth, high treatment costs, feed vaccination, and feed supplementations and accounts for 11.5%–29.5% of piglet deaths worldwide (Sinha *et al*., 2018; Wang *et al*., 2019). Therefore, the pig farming industry widely uses antibiotics to reduce *E. coli* infections and promote growth. However, the use of antibiotics, in addition to causing drug-resistant infections, also results in drug residues in the meat, putting the consumer at risk.

Swine production accounts for a large proportion of the global meat production (Österberg *et al*., 2016). In China, with large-scale pig farms, β-lactam resistance genes are most prevalent in the eastern region (Li *et al*., 2021). In Beijing, *E. coli* resistance rates range between 4.05% and 97.64%, and resistance to tetracyclines, penicillin, and chloramphenicol is most common (Liu *et al*., 2022). In northern Thailand, the prevalence of *E. coli* in chicken and swine farms ranges from 36.8% to 47.6% (Hanson *et al*., 2002). In this context, herbal extracts are increasingly being used as antibiotic replacements for organic animal production. Different herbal extracts have shown antibacterial activity, such as pomegranate rind, guava leaves, cinnamon and sappan wood extract (Mith *et al*., 2014; Nirmal and Panichayupakaranant, 2015; Chukiatsiri *et al*., 2021).

Sappan is a medicinal plant belonging to the bean family (Fabaceae or Leguminosae), with the scientific name *Caesalpinia sappan* L. It is commonly found in tropical countries such as India, Sri Lanka, Myanmar, Laos, Vietnam, South China, and Thailand. Its phenolic ingredients such as brazilin (sappan red), xanthone, coumarin, chalcones, and flavonoids, such as tannin and saponin, are active ingredients (Srinivasan *et*

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*al*., 2012; Zhao *et al*., 2013). Brazilin is the main ingredient in the heartwood of *C. sappan* and inhibits the growth of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE), and multidrug-resistant *Burkholderia cepacia* (Xu and Lee, 2004). Protosappanins A (PsA) and B (PsB) can inhibit the growth of MRSA (Zuo *et al*., 2015) and *C. sappan* methanol showed antimicrobial activity and the potential to restore the effectiveness of β-lactam antibiotics against MRSA (Kim *et al*., 2004) and *Streptococcus pyogenes* (Kaur *et al*., 2016). In other studies, ethanolic wood extracts of *C. sappan* (L.) showed activity against *Pseudomonas aeruginosa*, *S. aureus*, *Salmonella typhi*, *Enterobacter aeruginosa*, *Candida albicans*, *E. coli, Acinetobacter baumannii*, and *Klebsiella pneumoniae* (Temrangsee *et al*., 2011; Srinivasan *et al*., 2012).

This study investigated the efficiency of *C. sappan* wood extract in the treatment of *E. coli* infection in diarrheal pigs by oral treatment compared with antibiotic treatment. Feed supplemented with 1% and 2% *C. sappan* wood extract was evaluated, and it was expected that the herbal extract could be used as a substitute for antibiotic use in organic pig production.

## Materials and Methods

### *Caesalpinia sappan wood extraction*

*Caesalpinia sappan* wood extraction followed the protocol of Settharaksa *et al* (2019) Briefly, one kilogram of *C. sappan* wood was mixed with 6 l of water and soaked for 30 minutes. Subsequently, the mixture was boiled for 30 minutes (95°C), allowed to cool down, and filtered through cotton wool. The pulp was boiled three times, and the extracts were pooled and analyzed for volume, pH, and percentage of soluble sucrose to get 3% brix. Subsequently, we added Cab-O-Sil at 1.25% w/v, followed by shaking. Drying was performed with a BUCHI Mini Spray Dryer B-290 (Serial no.: 1000278016), which has a spray nozzle hole size of 0.7 mm, at an air pressure of 40 mbar. The drying conditions were as follows: 170°C inlet temperature, 110°C outlet temperature, aspirator 100%, pump 25%, and two nozzle cleaners. The extract powder was kept in a vacuum-laminated sachet, and the packing date was recorded. Figure 1 shows images of the water extract and the powder.

### *Animal selection*

Diarrheal pigs from the sucking and weaning group for experiments 1 and 2 were selected on day 2 of showing the clinical signs and weight between 3 and 10 kg. Confirmation of *E. coli*-caused diarrhea was cultured according to Lupindu (2017).

Experiment 1 (direct oral treatment): 34 diarrheal weaned pigs were divided into two groups (17 pigs per group) that were orally treated with enrofloxacin 5 mg/kg and *C. sappan* wood extract 500 mg/kg.

Experiment 2 (feed treatment): diarrheal pigs were chosen from 5 l of suckling (36 pigs) and 5 l of weaning (36 pigs) on day 2 of showing clinical signs and weight between 3 and 5 kg. Both suckling and weaning piglets were divided into three groups, 12 diarrheal piglets per group. Group 1 was fed normal feed, and groups 2 and


**Fig. 1.** Images of *C. sappan* wood, wood extract, and wood powder.

3 were fed with 1% and 2% *C. sappan* wood extract mixed feed, respectively. The feeding process was 7 days starting from day 7 to 14 post-partum, four times per day and water ad libitum. The amount of feed per day, shape, and color of feces was recorded.

***Escherichia coli isolation and minimal inhibition concentration (MIC) test***

Isolates of *E. coli* were recovered from diarrheal piglets according to Lupindu (2017). Briefly, 1 g of feces was mixed with lauryl sulfate broth and incubated at 37°C for 18 to 24 hours. MacConkey agar (MAC) and eosin methylene-blue (EMB) lactose sucrose agar (EMB) were used to isolate *E. coli* colonies. Round, medium-sized colonies on MAC and metallic, sheen colonies on EMB were selected and spread on nutrient agar (NA), followed by incubation at 37°C for 18 to 24 hours. Single colonies on NA were tested for confirmation and kept as stock in tryptic soy broth + glycerol 2%. Confirmed isolates were recovered on NA and then cultured in Müller–Hinton broth. Incubation was done at 37°C for 18 to 24 hours, and absorbance was measured at 550 nm for 0.5 McFarland, which yielded an estimation of *E. coli at* $10^8$ CFU/ml, with a dilution to $10^7$ CFU/ml. Sappan wood extract was prepared by mixing with Müller–Hinton agar (MHA) at concentrations of 128, 64, 32, 16, 8, 4, 2, and 0 mg/ml. Subsequently, 2 µl of *E. coli* ($10^7$ CFU/ml) was dropped on MHA at four points and incubated at 37°C for 18 to 24 hours. After this, the MIC was determined. Antibiotic resistance was interpreted according to CLSI (2008) and BSAC (2011).

***Enumeration of E. coli in diarrheal pigs***

Briefly, 1 g of fecal sample was added to trypticase soy broth and diluted to obtain concentrations of $10^{-4}$ to $10^{-9}$. Then, 0.1 ml of each dilution was spread on EMB and cultured at 37°C for 18–24 hours. The colony number was determined and calculated per volume of sample (1 mg).

***Application of C. sappan for treating E. coli infection***

Feed treatment; supplemented feed was prepared by using *C. sappan* wood extract 1% and 2% with pig feed. Then, 1% and 2% mixed feed were fed diarrheal pigs group 1 and 2 of suckling and weaning pigs compared to the normal feed group. Fecal score and body weight were recorded daily (7 days). Diarrheal pigs were treated with *C. sappan* wood extract 500 mg/kg BW compared with those treated with enrofloxacin 5 mg/kg BW for 10 days.

***Fecal scoring***

The fecal score was evaluated in the early morning by visualization of the fecal consistency, shape, and color with the following scores: 1 = dark, firm, and bushy, 2 = semi-solid with black and green, 3 = relatively soft with greenish–yellow, 4 = rather liquid (either loose or formed) with greenish yellow and grey, 5 = watery diarrhea with yellow.

***Statistical analysis***

Data were collected from individual pigs, and the differences in the mean values between the group, standard deviation, and the *p*-value for the statistical difference were analyzed by using Microsoft Excel. Qualitative analysis of *E. coli* shedding and body weight were performed using compute paired samples Wilcoxon test in R. Ordinal data of fecal shape and fecal color were analyzed by using nonparametric statistical methods "Kruskal Wallis test" (Statistical Program R; Free Software Foundation, Boston, MA).

***Ethical approval***

This study was approved by the ethics committee of Maejo University *(*Approval no. MACUC 035A/2560).

## Results

The MIC of both water and ethanol *C. sappan* wood extract for inhibiting *E. coli* growth was 16–32 mg/ml (Table 1). The average colony forming unit (CFU) of *E. coli* at 5 days before treatment with enrofloxacin and sappan wood extract were $5.70 \times 10^8$ and $6.10 \times 10^8$ CFU/ml, respectively. After treatment for 5 days, *E. coli* of the antibiotic treatment group was $2.8 \times 10^7$ CFU/ml, whereas in the *C. sappan* wood extract treatment group was $2.6 \times 10^7$ CFU/ml. After 10 days of treatment, $1.69 \times 10^6$ and $1.59 \times 10^6$ CFU/ml in enrofloxacin and Sappan wood extract treatment groups, respectively.

**Table 1.** The MIC of *C. sappan* wood extract against *E. coli.*

| The concentration of *C. sappan* wood extract ((mg/ml) | *E. coli* isolates | | Percentage (%) |
|---|---|---|---|
| | Water extract | Ethanol extract | |
| 128 | - | - | - |
| 64 | - | - | - |
| 32 | 1 | 1 | 2 |
| 16 | 9 | 9 | 98 |
| 8 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 |
| Total isolates | 10 | | |

The average number of *E. coli* (CFU/ml) before and after treatment for 5 and 10 days did not significantly differ between the enrofloxacin and *C. sappan* wood treatment groups ($p > 0.05$) (Table 2).

Treating with sappan wood extract and enrofloxacin showed significantly improved fecal shape and fecal color ($p < 0.005$). The Sappan wood extract treatment group showed better improvement than the enrofloxacin treatment group ($p < 0.005$). Comparison within the sappan wood extract and enrofloxacin treating groups determined that there was no significant difference in the improvement of fecal score and fecal color ($p > 0.005$) (data not showed). The average body weights of piglets in the enrofloxacin treatment group at 5 days before treatment and 5 and 10 days after treatment were 3.67, 4.23, and 4.67 kg. In the sappan wood extract treatment group, at 5 days before treatment and 5 and 10 days after treatment, the average body weights were 3.72, 4.25, and 4.93 kg. However, there were no statistically significant differences ($p > 0.05$) between the two groups (Table 3).

Diarrheal-sucking pigs supplemented with 1% and 2% *C. sappan* wood extract feed, fecal shape differed significantly from the control group at days 1, 5, 6, and 7, whereas there were significant differences between treatments with 1% and 2% *C. sappan* wood extract ($p < 0.05$) at day 6. Comparison within each group from day 1 to 7, feeding with 1% sappan wood showed significantly better improvement of fecal shape at day 6 than on day 5. Feeding with 2% sappan wood showed significantly better improvement of fecal shape on days 6 and 7 than on day 5. The fecal color of the 1% and 2% *C. sappan* wood extract treatment groups differed significantly from that of the control group on days 4, 5, 6, and 7, but there was no significant difference within the group (Table 4).

The average daily gain (ADG) values of suckling pigs of the control group, the 1% feeding group, and the 2% feeding group did not significantly differ (data not showed). However, the feed intake of suckling pigs fed 1% (69.4 ± 25.1) and 2% (68.9 ± 24.1) was lower than that of the control group (92.2 ± 53.9).

In weaning pigs fed with 1% and 2% mixed feed, there were no differences in fecal shape, fecal score, and ADG when compared to the control group. Fecal color and score did not significantly differ between the enrofloxacin treatment group and the sappan wood extract treatment group from day 1 to day 10 ($p > 0.05$) (data not showed).

## Discussion

Enteric colibacillosis in swine is mostly caused by enterotoxigenic *E. coli*. This bacterium produces one or more enterotoxins that can have local and systemic effects, causing the secretion of fluid and electrolytes into the intestinal lumen, which results in diarrhea, dehydration, and acidosis (Castro *et al*., 2022). One of the antibiotics used for the treatment of swine colibacillosis is enrofloxacin, which is generally used for the treatment of gram-negative bacterial infections of the urinary and gastrointestinal tract.

Generally, *E. coli* isolated from pigs is most frequently resistant to many antibiotics. In northern Thailand, *E.coli* shows resistance to tetracycline (91.5%), nalidixic acid (67.4%), ampicillin (61.6%), florfenicol (51.8%), enrofloxacin (28.7%), ciprofloxacin (12.5%), ceftiofur (4.9%), and ceftriaxone (1.5%) (Hanson *et al*., 2002). Pathogenic *E. coli* isolated from diarrheal pigs in Thailand resisted multidrugs such as novobiocin, streptomycin, sulfamethoxazole, tetracyclin, and tiamulin (100%), followed by amoxicillin (98%), oxytetracycline (96%), nalidixic acid (82%), gentamicin (56%), enrofloxacin (54%), and Colistin sulfate (46%) but susceptible to (Chukiatsiri *et al*, 2021). While another study indicated that, *E. coli* isolates from diarrheal piglets showed highly resistant

**Table 2.** Average numbers of *E. coli* at 5 days before treatment and 5 and 10 days after treatment.

| | $T_1$ (CFU/ml) | $T_2$ (CFU/ml) | *p* values |
|---|---|---|---|
| 5 days before treatment | 5.45 ± 6.16 Î $10^8$ | 5.83 ± 5.27 Î $10^8$ | 0.914 |
| 5 days after treatment | 2.47 ± 3.76 Î $10^8$ | 2.35 ± 3.5 Î $10^8$ | 0.914 |
| 10 days after treatment | 1.69 ± 2.35 Î $10^8$ | 1.47 ± 2.34 Î $10^8$ | 0.944 |

$T_1$: Enrofloxacin (5 mg/kg BW) treatment group; $T_2$: Sappan wood extract (500 mg/kg) treatment group.

**Table 3.** Average body weights of piglets before treatment and 5 and 10 days after treatment.

| | $T_1$ (kg) | $T_2$ (kg) | *p* values |
|---|---|---|---|
| 5 days before treatment | 3.67 ± 0.96 | 3.72 ± 0.92 | 0.921 |
| 5 days after treatment | 4.23 ± 0.96 | 4.25 ± 0.93 | 0.973 |
| 10 days after treatment | 4.67 ± 0.98 | 4.93 ± 0.98 | 0.479 |

$T_1$: Enrofloxacin (5 mg/kg BW) treatment group; $T_2$: sappan wood extract (500 mg/kg) treatment group.

**Table 4.** Scoring of fecal shape and color in suckling pigs fed with 1% (T1) and 2% (T2) *C. sappan* wood extract feed compared with the control group (normal feed).

| | Day | Control | T1 | T2 |
|---|---|---|---|---|
| Faecal shape | 1 | 1.73 ± 0.18 | 1.30 ± 0.10* | 1.53 ± 0.08* |
| | 2 | 2.03 ± 0.32 | 1.73 ± 0.13 | 1.69 ± 0.20 |
| | 3 | 2.16 ± 0.20 | 2.16 ± 0.18 | 2.06 ± 0.08 |
| | 4 | 2.96 ± 0.34 | 2.26 ± 0.13 | 2.10 ± 0.11 |
| | 5 | 3.06 ± 0.12 | 2.20 ± 0.00* | 2.20 ± 0.05* |
| | 6 | 3.00 ± 0.15 | 1.76±0.08*ab | 2.16 ± 0.23*ab |
| | 7 | 3.06 ± 0.06 | 1.90 ± 0.20* | 1.90 ± 0.15*b |
| Fecal color | 1 | 1.96 ± 0.03 | 2.13 ± 0.23 | 2.40 ± 0.26 |
| | 2 | 2.80 ± 0.10 | 2.46 ± 0.06 | 2.53 ± 0.03 |
| | 3 | 2.93 ± 0.26 | 2.50 ± 0.15 | 2.60 ± 0.10 |
| | 4 | 3.23 ± 0.12 | 2.80 ± 0.10* | 2.63 ± 0.08* |
| | 5 | 3.56 ± 0.03 | 2.63 ± 0.08* | 2.63 ± 0.12* |
| | 6 | 3.60 ± 0.20 | 2.70 ± 0.10* | 2.46 ± 0.32* |
| | 7 | 3.53 ± 0.08 | 2.43 ± 0.08* | 2.20 ± 0.05* |

*significant difference between experiment groups (T1, T2) and the control group ($p < 0.05$); [a]significant difference between T1 and T2 group ($p < 0.05$); [b]significant difference within-group ($p < 0.05$).

to amoxicillin (100%), followed by oxytetracycline (91.9%), enrofloxacin (89.2%), trimethoprim/sulfamethoxazole (86.5%), amoxicillin: clavulanic acid (81.1%), colistin and gentamicin (75.7%), ceftriaxone and ceftiofur (64.9%), and ceftazidime (35.1%); 97.3% showed multidrug resistance (Nguyet *et al*., 2022). The use of enrofloxacin in piglets become increases the odds for enrofloxacin resistance in piglets (OR = 26.78; $p \leq 0.0001$) and sows at weaning (OR = 4.04; $p \leq 0.05$) (Callens *et al*., 2015). Resistance of *E. coli* to enrofloxacin has been reported in many species of livestock such as cattle, poultry included swine (Lin *et al*., 2017; Astorga *et al*., 2019; Li *et al*., 2019).

This study used the optimal conditions that provide the highest active ingredient (brazilin) content by extraction at a temperature of 95°C for 30 minutes, as suggested by Settharaksa (2019). These results indicate that *C. sappan* wood extract can inhibit the growth of *E. coli*, (MIC: 16–32 mg/ml). The MIC obtained in the present study was lower than that reported in a study that determined the antibacterial activity of *C. sappan* wood extract against foodborne pathogens, including *S. aureus*, *E. coli*, *S. enteritidis,* and *V. parahaemolyticus*, with a MIC of 200 mg/ml (Pattananandecha *et al*., 2022). But it showed higher than a previous which indicated that ethanolic extracts of *C. sappan* wood extract can be used against bacteria that cause chronic wound infection, such as *S. aureus*, with a MIC value of 0.625 mg/ml (Temrangsee, 2011) and MIC/MBC 2/2 mg/ml (Kawari *et al*, 2016). The Brasilin which used a bioassay-directed method for isolating showed a potential activity against antibiotic-resistant bacteria at the MIC 4–32 mg/ml (Xu and Lee, 2004).

For treating E. *coli*-caused diarrheal, this study was designed to use *C. sappan* wood extract 500 mg/kg BW because of the unestimated number of bacteria in the gut of diarrheal pigs. The results determined that the efficiency of *C. sappan* wood extract by oral route treatment of *E. coli* did not significantly differ from that of enrofloxacin. Moreover, the average body weights of piglets treated with enrofloxacin and *C. sappan* wood extract showed similar. Indicated that *C. sappan* wood extract did not improve the growth rate of piglets.

Feeding with 1% sappan wood extract mixed feed showed a higher improvement of fecal shape at day 6 after treatment than that of the 2% treatment and the control group. However, feed intake and ADG were lower than in the control group, most likely because of the bitter taste of sappan wood extract. Using sappan wood extract (1% and 2%) mixed with feed did not improve diarrheal signs in weaned pigs. Similar to the results of a previous study which indicated that the flavonoids in *C. sappan* extract can decrease the number of *Salmonella*, but not of *E. coli*, in quail intestines (Widigdyo *et al*., 2017).

The results of the present study suggest that *C. sappan* wood extract can be used as an alternative in the treatment of *E. coli* infections in sucking pigs at a concentration of 1%, *C. sappan* wood extract supplemented in feed. These results showed that although treatment with *C. sappan* decreases the CFU/ml of *E. coli;* it does not show better efficiency with respect to enrofloxacin treatment. The potential biases or limitations inherent in the study design were a bitter test of sappan wood extract and animal death during the experiment.

**Acknowledgment**

The research team would like to thank the staff at the Faculty of Animal Science and Technology, Maejo University, for their support. Thanks are due to Mr. Polkrit Vijitpongsa, Mr. Jatupohn Tongkumheo, Mr. Nattapong Tonksak, and Miss Natkritta Kongkart for providing assistance in the experiments for this research. This project was not supported by any grants.

***Authors' contributions***

Kridda Chukiatsiri: Study design, sampling, diagnosis, and analysis of the raw data. Kittiphong Tippaya: Sampling, diagnosis, and analysis of the raw data. Ruttayaporn Ngasaman: Analyzed the data and writing the manuscript.

***Conflict of interest***

The authors declare that there is no conflict of interest.

***Funding***

Maejo University funding.

***Data availability***

All data are provided in the manuscript.

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