

The antibacterial potential of curly red chili pepper (*Capsicum annuum* L.) fruit stem waste against foodborne disease bacteria

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Abstract: Foodborne diseases remain a significant global public health concern, frequently associated with pathogenic bacteria such as *Salmonella typhi*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*. The exploration of natural, sustainable resources as alternative antibacterial agents has gained increasing attention, particularly the valorization of agricultural waste. This study aimed to evaluate the antibacterial potential of curly red chili pepper (*Capsicum annuum* L.) fruit stem waste extract. The plant waste was extracted using maceration with 96% ethanol as the solvent. Phytochemical screening revealed the presence of bioactive compounds, including flavonoids, saponins, tannins, alkaloids, steroids, and phenolics, which are commonly associated with antimicrobial activity. Antibacterial activity was assessed using the disc diffusion method at concentrations of 70%, 80%, 90%, and 100%, with ciprofloxacin as the positive control and distilled water as the negative control. The results demonstrated that all extract concentrations produced inhibition zones classified as “resistant” according to CLSI standards, indicating relatively weak antibacterial activity. The highest inhibition was observed at 100% concentration, with greater susceptibility in Gram-positive bacteria (*B. cereus* and *S. aureus*) compared to Gram-negative bacteria (*S. typhi* and *E. coli*). This difference may be attributed to structural variations in bacterial cell walls that influence permeability to bioactive compounds. In conclusion, curly red chili pepper fruit stem extract exhibits limited antibacterial efficacy under the tested conditions. Nevertheless, its phytochemical content suggests potential for further optimization through extraction methods, concentration enhancement, or formulation strategies, supporting its prospective application as a value-added product in sustainable and natural antimicrobial development.

Keywords: *antibacterial, Capsicum annuum L., foodborne diseases, secondary metabolites*

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INTRODUCTION

Foodborne diseases are conditions caused by the consumption of food or beverages contaminated with pathogenic microorganisms. The clinical manifestations of foodborne diseases vary depending on the type of pathogen and the infected individual. One of the most common foodborne diseases is diarrhea (Muna, 2020). The World Health Organization (WHO) (2024) reported that diarrhea is the third leading cause of death worldwide, accounting for approximately 600 million cases (30%) and resulting in about 420,000 deaths among children under five years of age. The primary factors contributing to diarrhea are infections caused by various bacteria, including *Salmonella typhi*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* (Huang et al., 2016).

According to the Indonesian Health Survey in 2023, the prevalence of diarrhea across all age groups was 2%, with a prevalence of 4.9% among toddlers and 3.9% among infants. In the same year,

the coverage of diarrhea treatment services reached 41.5% for all age groups and 31.7% for toddlers, based on established targets (Ministry of Health of the Republic of Indonesia, 2023). In Lampung Province in 2024, the number of treated diarrhea cases reached 5,754 individuals (Central Bureau of Statistics of Bandar Lampung City, 2024).

One of the primary treatments for bacterial-induced diarrhea is the use of antibiotics. Antibiotics have long been the mainstay of infection management; however, their excessive or prolonged use may lead to antibiotic resistance. In cases of antibiotic resistance, the development of alternative treatments using natural products is necessary. One such potential source is curly red chili pepper (*Capsicum annum* L.). This plant exhibits effective antibacterial activity against bacterial growth, which is attributed to the presence of secondary metabolites identified through phytochemical screening, including alkaloids, flavonoids, tannins, and saponins.

Previous studies have reported antibacterial activity using the disc diffusion method, with inhibition zones of 8.56 mm against *Escherichia coli* and 3.55 mm against *Staphylococcus aureus* at a concentration of 100% (Ananta & Anjasmara, 2022). Agnes (2024) reported inhibition zones of 6.7 mm against *Escherichia coli* and *Salmonella typhi* at the same concentration. Meanwhile, Grojja (2023) reported inhibition zones of 11.66 mm against *Escherichia coli* and 9.00 mm against *Bacillus cereus*. Similarly, Sapitri et al. (2021) demonstrated that the ethanol extract of red chili pepper (*Capsicum annum* L.) contains various secondary metabolites and exhibits antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with inhibition zones increasing proportionally to the extract concentration.

However, previous studies have primarily focused on chili fruit, while the antibacterial potential of chili fruit stem waste remains poorly understood, with limited evidence regarding its effectiveness against clinically relevant foodborne pathogens. Although research on chili pepper fruit has been widely conducted, studies specifically examining the antibacterial activity of chili pepper fruit stems are still scarce, and the stems are generally regarded as domestic waste. Nevertheless, curly red chili pepper (*Capsicum annum* L.) fruit is known to contain secondary metabolites with antibacterial properties. Given the direct physiological connection between the fruit and its stem, it is plausible that the stem also contains bioactive compounds with antibacterial activity. Therefore, this study aimed to evaluate the potential of curly red chili pepper (*Capsicum annum* L.) fruit stem waste extract as a natural antibacterial agent.

METHOD

The methods section should contain approximately 10–15% of the total manuscript and include the research design, research subjects or materials, instruments, data collection procedures, and data analysis techniques. The manuscript should be written using Times New Roman font size 11 pt, single spacing, and justified alignment. Scientific names and foreign terms should be written in italics.

Instruments and Materials

The instruments used in this study included test tube racks, test tubes, graduated pipettes, glass stirring rods, Erlenmeyer flasks, beakers, inoculating loops, gloves, spatulas, blenders, forceps, Petri dishes, paper discs, masks, a rotary vacuum evaporator, sterile cotton, coffee filter paper, a Bunsen burner, funnels, a zone reader, micropipettes, an incubator, an oven, a vernier caliper, a showcase refrigerator, an analytical balance, and an autoclave.

The materials used consisted of curly red chili pepper fruit stem waste collected from Pasir Gintung Market, Bandar Lampung City; 96% ethanol; 0.9% NaCl solution; Nutrient Agar (NA); Mueller–Hinton Agar (MHA); McFarland standard; 1% BaCl₂; 1% H₂SO₄; bacterial isolates of *Salmonella typhi*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*; ciprofloxacin as a positive control; and distilled water as a negative control.

Plant Determination and Sample Preparation

The first stage of the study involved taxonomic identification of the plant material, followed by simplisia preparation through wet and dry sorting. Wet sorting was performed by weighing 1.5 kg of chili pepper fruit stems, washing them under running water, cutting them into two parts, and drying

them under direct sunlight. To accelerate the drying process, the samples were covered with black cloth, which is believed to absorb heat effectively. This method is consistent with the findings of Sartini et al. (2017), who reported that drying under black cloth resulted in slower oxidation and minimal contamination. The dried samples were further sorted, ground using a blender, sieved, and stored in a closed container at room temperature.

Preparation and Extraction of Curly Red Chili Pepper Fruit Stems

Extraction of the fruit stems was performed using the maceration method with 96% ethanol as the solvent. A total of 800 g of powdered sample was placed into a maceration container and soaked in 8,000 mL of solvent. The maceration process was carried out for 3×24 hours. The resulting filtrate was concentrated using a rotary evaporator at 40°C to obtain a thick extract, and the extraction yield was subsequently calculated. The yield was considered satisfactory if it exceeded 10% (Ananta & Anjasmara, 2022).

Phytochemical Screening

Phytochemical screening was conducted to identify the presence of flavonoids, saponins, tannins, alkaloids, terpenoids, steroids, and phenolic compounds.

Flavonoid Test

A total of 0.5 mL of the extract was heated until boiling. Magnesium powder (0.5 g) and 0.5 mL of hydrochloric acid were added to the sample. A positive result was indicated by the appearance of red, yellow, or orange coloration.

Saponin Test

A volume of 0.5 mL of the extract was mixed with 5 mL of distilled water, heated for 5 minutes, and homogenized for 30 seconds. The formation of stable foam indicated a positive result for saponins.

Tannin Test

One milliliter of the extract was added with three drops of 10% FeCl₃ solution. The appearance of bluish-black or greenish-black coloration indicated the presence of tannins.

Alkaloid Test

A total of 0.5 mL of the extract was added with five drops of chloroform and three drops of Mayer's reagent. A positive result was indicated by the formation of orange or brown coloration accompanied by a white precipitate.

Terpenoid Test

A volume of 0.5 mL of the extract was mixed with 0.5 mL of glacial acetic acid and 0.5 mL of concentrated sulfuric acid. The appearance of red, purplish-red, or yellow coloration indicated the presence of terpenoids.

Steroid Test

A total of 0.5 mL of the extract was mixed with 0.5 mL of glacial acetic acid and 0.5 mL of concentrated sulfuric acid. A positive result was indicated by blue, purple, or greenish coloration.

Phenolic Compound Test

One milliliter of the extract was added with three drops of 2% FeCl₃ reagent. The formation of bluish-black coloration indicated the presence of phenolic compounds.

Preparation of Nutrient Agar (NA) and Mueller–Hinton Agar (MHA)

Antibacterial testing was conducted using NA and MHA media. NA was prepared by dissolving 0.68 g of powder in 20 mL of distilled water, while MHA was prepared by dissolving 16.32 g of powder in 480 mL of distilled water. The media were sterilized in an autoclave at 121°C and 2 atm pressure for 15 minutes, cooled to 45–50°C, and allowed to solidify at a 30° inclination (Hasani et al., 2024).

Bacterial Rejuvenation

Bacterial rejuvenation was performed by aseptically pouring 5 mL of molten Nutrient Agar into test tubes and allowing it to solidify. Pure cultures of *Salmonella typhi*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* were inoculated onto the respective media using a sterile inoculating

loop. The cultures were then incubated at 37°C for 24 hours at room temperature (Aronggera, 2022). The details of antibacterial activity testing are presented in Table 1.

Table 1. Details of antibacterial activity testing of curly red chili pepper fruit stem extract

Treatment for <i>Salmonella typhi</i>	Treatment for <i>Escherichia coli</i>	Treatment for <i>Bacillus cereus</i>	Treatment for <i>Staphylococcus aureus</i>
Pst 1 (100%)	Pec 1 (100%)	Pbc 1 (100%)	Psa 1 (100%)
Pst 2 (90%)	Pec 2 (90%)	Pbc 2 (90%)	Psa 2 (90%)
Pst 3 (80%)	Pec 3 (80%)	Pbc 3 (80%)	Psa 3 (80%)
Pst 4 (70%)	Pec 4 (70%)	Pbc 4 (70%)	Psa 4 (70%)
Pst 5 (antibiotic)	Pec 5 (antibiotic)	Pbc 5 (antibiotic)	Psa 5 (antibiotic)
Pst 6 (distilled water)	Pec 6 (distilled water)	Pbc 6 (distilled water)	Psa 6 (distilled water)

Notes:

- a) Pst 1, Pec 1, Pbc 1, Psa 1: Treatment at 100% extract concentration
- b) Pst 2, Pec 2, Pbc 2, Psa 2: Treatment at 90% extract concentration
- c) Pst 3, Pec 3, Pbc 3, Psa 3: Treatment at 80% extract concentration
- d) Pst 4, Pec 4, Pbc 4, Psa 4: Treatment at 70% extract concentration
- e) Pst 5, Pec 5, Pbc 5, Psa 5: Positive control (ciprofloxacin antibiotic)
- f) Pst 6, Pec 6, Pbc 6, Psa 6: Negative control (distilled water)

Antibacterial Activity Assay

Antibacterial activity testing was performed by inoculating 1 mL of bacterial suspension onto Mueller–Hinton Agar (MHA). Paper discs impregnated with distilled water, antibiotic solution, and curly red chili pepper fruit stem extract at concentrations of 100%, 90%, 80%, and 70% were placed onto the agar surface. The extract concentrations used in this study were higher than those reported by Agnes (2024), who employed concentrations of 100%, 75%, 50%, and 25%, in order to anticipate higher levels of bacterial resistance and to ensure that antibacterial activity could be clearly observed on the MHA medium. All tests were performed in triplicate. The plates were incubated at 37°C for 24 hours, after which the inhibition zones were observed and the diameters of the inhibition zones were measured.

Data Analysis

The observed data were analyzed qualitatively and quantitatively. Quantitative data were processed using SPSS software version 26, including normality, homogeneity, and bivariate tests.

FINDINGS AND DISCUSSION

Extraction of Curly Red Chili Pepper Fruit Stems

Extraction of curly red chili pepper fruit stems was carried out using the maceration method. A total of 800 g of fruit stem sample was extracted using 8,000 mL of 96% ethanol as the solvent. The thick extract obtained weighed 64.87 g, yielding an extraction yield of 8.1%. A previous study by Agnes (2024) reported a higher yield of 10%. Differences in extraction yield may be influenced by variations in the amount of simplicia and extraction duration. Agnes (2024) used 1,000 g of simplicia with a maceration period of five days, whereas the present study employed a maceration time of 3 × 24 hours, which may account for the lower yield obtained.

Phytochemical Screening of Curly Red Chili Pepper Fruit Stem Extract

The results of phytochemical screening of curly red chili pepper (*Capsicum annum L.*) fruit stem extract are presented in Table 2.

Table 2. Phytochemical screening of curly red chili pepper fruit stem extract

Test	Observation Result	Interpretation
Flavonoid	Yellow solution with foam formation	Positive (+)
Saponin	Stable foam formation	Positive (+)
Tannin	Greenish-black coloration	Positive (+)
Alkaloid	Yellowish-brown coloration	Positive (+)
Terpenoid	Dark green coloration	Negative (-)
Steroid	Bluish-green coloration	Positive (+)
Phenolic	Bluish-black coloration	Positive (+)

The results indicated the presence of flavonoids, saponins, tannins, alkaloids, steroids, and phenolic compounds, while terpenoids were not detected. The presence of flavonoids was confirmed by a yellow color change, which, according to Hidayati et al. (2020), results from the reaction between magnesium powder and concentrated HCl that reduces the benzopyrone nucleus, forming flavilium salts with red or orange coloration. The saponin test yielded positive results, indicated by stable foam formation. This phenomenon occurs because glycosides possess foaming properties in aqueous solutions and undergo hydrolysis into glucose (Ananta & Anjasmara, 2022). The tannin test showed a positive result with a greenish-black color change due to complex formation between Fe^{3+} ions and phenolic groups in the tannin structure (Hidayati et al., 2020). Alkaloid detection showed a positive reaction characterized by brownish coloration, which is attributed to reactions between chloroform and Mayer's reagent (KI and $HgCl_2$), where nitrogen atoms in alkaloids interact with potassium ions (K^+) from potassium tetraiodomercurate (II) to form alkaloid-potassium complexes (Rubianti et al., 2022). The terpenoid test yielded negative results, possibly due to the very low concentration of terpenoids, making them undetectable. Steroid compounds showed positive results with bluish-green coloration resulting from reactions involving glacial acetic acid and concentrated sulfuric acid (Nurjannah et al., 2022). Phenolic compounds were confirmed by bluish-black coloration caused by complex formation between Fe^{3+} ions and aromatic hydroxyl groups in phenolic structures (Asmah et al., 2020).

Alcohol-Free Test

The results of the alcohol-free test of curly red chili pepper fruit stem extract are shown in Table 3.

Table 3. Alcohol-free test results

Sample	Test Parameter	Result	Description
Curly red chili pepper fruit stem extract	Alcohol-free test	Positive (+)	Alcohol-free (no ester odor detected)

The alcohol-free test confirmed that the extract used in antibacterial activity testing was free from residual alcohol, as no ester odor was detected. This indicates the absence of reactions between carboxylic acids and alcohols. The alcohol-free test is essential to ensure that antibacterial activity is attributed to plant secondary metabolites rather than the antiseptic properties of alcohol, thereby preventing false-positive results in antibacterial assays (Sukadiasa et al., 2023).

Antibacterial Activity

The results of antibacterial activity testing of curly red chili pepper fruit stem extract are presented in Table 4.

Table 4. Antibacterial activity of curly red chili pepper fruit stem extract

Bacteria	Concentration	Inhibition Diameter (mm)			Zone Mean Zone \pm SD (mm)	Inhibition Growth Category
		P1	P2	P3		
<i>Salmonella typhi</i>	100%	0.40	0.70	0.80	0.63 \pm 0.20	Resistant
	90%	0.30	0.20	0.56	0.35 \pm 0.18	Resistant
	80%	0.20	0.13	0.50	0.27 \pm 0.19	Resistant
	70%	0.20	0.10	0.26	0.18 \pm 0.08	Resistant
	Positive control (Ciprofloxacin)	11.7	13.4	13.3	12.8 \pm 0.95	Intermediate
	Negative control (Distilled water)	0	0	0	0 \pm 0	Not detected
<i>Escherichia coli</i>	100%	0.43	0.36	1.10	0.63 \pm 0.40	Resistant
	90%	0.16	0.30	0.60	0.35 \pm 0.22	Resistant
	80%	0.13	0.20	0.53	0.28 \pm 0.21	Resistant
	70%	0.10	0.10	0.46	0.22 \pm 0.20	Resistant
	Positive control (Ciprofloxacin)	26.5	27.6	25.3	26.46 \pm 1.15	Susceptible
	Negative control (Distilled water)	0	0	0	0 \pm 0	Not detected
<i>Bacillus cereus</i>	100%	0.53	1.06	1.20	0.93 \pm 0.35	Resistant
	90%	0.23	0.90	0.83	0.65 \pm 0.36	Resistant
	80%	0.20	0.76	0.46	0.47 \pm 0.28	Resistant
	70%	0.10	0.43	0.30	0.27 \pm 0.16	Resistant
	Positive control (Ciprofloxacin)	18.2	24.2	29.8	24.06 \pm 5.80	Susceptible
	Negative control (Distilled water)	0	0	0	0 \pm 0	Not detected
<i>Staphylococcus aureus</i>	100%	1.76	0.40	0.60	0.92 \pm 0.73	Resistant
	90%	1.40	0.20	0.36	0.65 \pm 0.65	Resistant
	80%	1.06	0.13	0.23	0.47 \pm 0.51	Resistant
	70%	0.20	0.10	0.13	0.14 \pm 0.05	Resistant
	Positive control (Ciprofloxacin)	22.4	20.5	22.5	21.8 \pm 1.12	Susceptible
	Negative control (Distilled water)	0	0	0	0 \pm 0	Not detected

The antibacterial activity test against *Salmonella typhi*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* at extract concentrations of 100%, 90%, 80%, and 70% consistently produced inhibition zones categorized as “resistant.” These findings indicate that the extract of curly red chili pepper (*Capsicum annuum* L.) fruit stem exhibits relatively weak antibacterial activity under the tested conditions. This result contrasts with the study by Agnes (2024), which reported no antibacterial activity at lower concentrations (25–75%) but observed moderate inhibition at 100% concentration. The

discrepancy may be attributed to differences in extraction efficiency, solvent polarity, phytochemical composition, and experimental conditions, all of which are known to influence antimicrobial performance.

The inhibition zones produced by the positive control (ciprofloxacin) against *S. typhi*, *E. coli*, *B. cereus*, and *S. aureus* were 12.8 mm, 26.46 mm, 24.06 mm, and 21.8 mm, respectively, indicating strong to very strong antibacterial activity. In contrast, the negative control (distilled water) showed no inhibition, confirming that the observed antibacterial effects were attributable to the active agents. Ciprofloxacin is a broad-spectrum fluoroquinolone antibiotic that acts by inhibiting DNA gyrase and topoisomerase IV, thereby disrupting bacterial DNA replication and transcription (Mardina et al., 2021). Its effectiveness against both Gram-positive and Gram-negative bacteria in this study is consistent with established CLSI standards and previous findings.

Statistical analysis further revealed that, for *S. typhi*, no significant differences ($p > 0.05$) were observed among extract concentrations and the positive control, while the negative control differed significantly from all treatments. A similar pattern was observed for *E. coli* and *B. cereus*, where extract treatments did not differ significantly from the positive control but were significantly different from the negative control. Interestingly, for *S. aureus*, no significant differences were observed across all treatments, including the negative control, suggesting high variability or limited sensitivity of this bacterium under the experimental conditions. These results imply that although the extract exhibits measurable antibacterial effects, its efficacy is not statistically comparable to standard antibiotics.

The relatively weak antibacterial activity observed in this study may be explained by the limited diffusion capacity of bioactive compounds in the agar medium, as well as suboptimal concentrations of active constituents. Although phytochemical screening confirmed the presence of flavonoids, phenolics, saponins, tannins, and alkaloids, the concentration and bioavailability of these compounds play a critical role in determining antimicrobial efficacy. Previous studies have demonstrated that phenolic and flavonoid compounds exert antibacterial activity by disrupting bacterial cell membranes, increasing permeability, and causing leakage of intracellular components (Shang et al., 2019; Rather et al., 2017). Saponins, on the other hand, are known to interact with membrane sterols, leading to cell lysis and death (Dong et al., 2020). However, insufficient extraction or low compound concentration may result in sub-inhibitory effects, as observed in this study.

Recent studies indexed in Scopus also support these findings, highlighting that plant-based extracts often exhibit weaker antibacterial activity in crude form but can be significantly enhanced through optimization strategies. For instance, Foods reported that garlic peel extracts showed enhanced antimicrobial activity after fractionation and concentration of phenolic compounds (Kim et al., 2022). Similarly, research published in Food Chemistry demonstrated that extraction methods and solvent selection critically affect the yield of bioactive compounds and their antibacterial potency (Lu et al., 2011). These studies suggest that the low activity observed in the present research may be improved through advanced extraction techniques, purification, or formulation approaches.

CONCLUSION

Based on the results and discussion, it can be concluded that the 100% extract concentration exhibited the largest inhibition zones. The mean inhibition zones against *Bacillus cereus* and *Staphylococcus aureus* (Gram-positive bacteria) were greater than those observed against Gram-negative bacteria, namely *Salmonella typhi* and *Escherichia coli*. These findings indicate that curly red chili pepper fruit stem extract possesses weak antibacterial potential and requires further investigation.

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