Antibacterial Activity of Coptis Rhizome Extracts Against *Micrococcus luteus* Using Different Extraction Methods

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Abstract
Coptis rhizome (Huang Lian) is the dried underground stem part of a plant called *Coptis chinensis* (Chinese goldthread), known to contain bioactive compounds with antibacterial potential, particularly berberine. This study investigated the antibacterial activity of Coptis rhizome extracts against *Micrococcus luteus*, a gram-positive bacterium that can cause opportunistic infections in immunocompromised individuals. Three extraction methods including aqueous, ethanol, and enzyme-assisted extraction (EAE), were evaluated to identify the approach with the strongest antibacterial activity. Based on the results obtained, aqueous extraction demonstrated the highest antibacterial activity against *M. luteus* among the three methods tested. This indicates that aqueous extraction is the most effective method for extracting antibacterial compounds from Coptis rhizome under the conditions of this study.

***Keywords*:** Coptis rhizome, *M. luteus,* aqueous extraction, ethanol extraction, enzyme-assisted extraction, antibacterial activity

I. INTRODUCTION

Coptis rhizome, also known as Huang Lian in Chinese, is a part of the *Coptis chinensis* plant commonly used for its pharmacological properties to treat various diseases such as bacillary dysentery and typhoid.[1] The herb has historical evidence of usage dating back thousands of years due to its functions of clearing heat, drying dampness, and detoxification according to the traditional Chinese Medicinal theory.

Studies have shown that Coptis rhizome consists of multiple secondary metabolites such as alkaloids, lignans, phenylpropanoids, flavonoids, phenolic acids, saccharides, and steroids. The main bioactive components in Coptis rhizome are berberine, palmatine, coptisine, epiberberine, jatrorrhizines, and columamine, with berberine[1] being the most important active constituent.

According to the existing research, berberine can destroy or degrade some of the bacteria's proteins that could lead to bacterial death. Additionally, berberine can inhibit DNA synthesis in bacteria by inhibiting the activity of DNA topoisomerase, which has the functions of untangling DNA during processes like DNA replication, transcription, and cell division.[2]

M. luteus was used to test the antibacterial properties of Coptis rhizome. *M. luteus* is a gram-positive, nonpathogenic bacterium that is commonly found in environments including soil, water, and even on the skin of animals and humans. Although this bacterium is generally considered nonpathogenic, *M. luteus* can cause infections in those with weakened immune systems, while posing minimal risk in healthy individuals.[3]

This experiment was carried out using nonpathogenic strains of *M. luteus* and appropriate biosafety procedures, which are considered safe for both the researchers and the environment.[4] Three extraction methods were used to extract antibacterial compounds from Coptis rhizome to test its antibacterial properties against *M. luteus:* aqueous extraction, ethanol extraction, and enzyme-assisted extraction (EAE).

In the aqueous extraction assay, Coptis rhizomes tissues were penetrated by water through the cell walls. This allowed water-soluble compounds, including berberine to leach out of the cells. The assay also involved heating, as heat weakened the bonds in cellulose and hemicellulose within the Coptis rhizome, resulting in membrane rupture.

Consequently, stored metabolites and bioactive compounds were released from the vacuoles. Nevertheless, most compounds were thermally stable; therefore, heating at this temperature did not degrade the bioactive compounds in the herb.[5]

In the ethanol extraction assay, ethanol was selected as the primary solvent because its hydroxyl (-OH) functional group enabled effective extraction of bioactive compounds. In addition, its relatively low toxicity compared with other alcohols[6] made it suitable for modeling the extraction of medicinal compounds from Coptis rhizome intended for human consumption. Ethanol also exhibited antibacterial activity by interacting with and destabilizing bacterial lipid membranes and denaturing proteins, thus increasing membrane permeability and causing leakage of essential intracellular components, ultimately leading to cell death.[7] This antibacterial mechanism enhanced the overall antimicrobial activity observed in alcohol Coptis rhizome extracts, particularly because ethanol efficiently extracted alkaloids such as berberine and related bioactive compounds, whose antibacterial potential increased with higher ethanol concentrations.[8]

In the EAE assay, limited research has been conducted on the extraction of bioactive compounds from Coptis rhizome. In this method, the most commonly used enzymes are cellulase, hemicellulase, and pectinase. A major advantage of this method is its selectivity. The process functions through the use of specific enzymes that hydrolyze the cell wall by forming enzyme-substrate binding complexes. This process requires specific conditions, including reaction temperature, system pH, enzyme concentration, and extraction time. Coptis rhizome consists of cell walls, which are primarily composed of cellulose fibers; therefore, cellulase was used in this experiment. Although this method offers advantages such as selective extraction and environmental friendliness, it is relatively expensive due to the cost of enzymes, and the extraction process may be time-consuming.[9]

The objective of this study was to compare the antibacterial effectiveness of the three extraction methods, against *M. luteus.* The effectiveness of each method was evaluated by comparing the diameters of the resulting zones of inhibition to determine which extraction method was the most effective.

II. METHODS

Materials and Preparation of Bacterial Culture

Coptis rhizome was sourced from a commercial supplier for use in the extraction process. A broth culture of *M. luteus* was sourced from the Thailand Institute of Scientific and Technological Research (TISTR). The broth culture was stored in a laboratory incubator at 27°C. The culture was then spread onto 30 agar plates and incubated at 27°C for 48 hours.[10]

A total of 14g of nutrient agar were mixed with 500 mL of distilled water and heated at 121°C for 15 minutes. The cooled agar was then poured into 30 sterile Petri dishes and left at room temperature (25°C) to solidify. Each plate was divided into four sections and grouped into three sets of 10 plates.[11] Each of the three extraction methods was tested using 10 agar plates (n = 10). In the aqueous extraction assay, Aq1, Aq2, and Aq3 represented aqueous-treated samples, while C represented the distilled water control. In the ethanol extraction assay, E1 and E2 represented ethanol-treated samples, Eth represented the ethanol control, and C represented the distilled water control. In the enzyme-assisted extraction (EAE) assay, EA1, EA2, and EA3 represented EAE-treated samples, while C represented the distilled water control. Only two ethanol-treated samples were tested per agar plate, unlike the other extraction methods, because the ethanol extraction method required both an ethanol control and a distilled water control. Per one agar plate, a maximum of four discs was limited to prevent the zones of inhibition from overlapping. Therefore, only two ethanol-treated samples could be tested, whereas the other extraction methods contained three treated samples per plate.

The *M. luteus* broth was pipetted onto each plate using aseptic technique, and a flame-sterilized glass spreader was used to distribute the broth evenly across the agar's surface.

Extraction Methods

In aqueous extraction, 10g of Coptis rhizome were prepared by crushing the roots into a powder and mixing them with 40 mL of distilled water. The ratio of dried material to water was 4:1.[12] The mixture was heated to 90 °C for 20 minutes before being filtered. Blank discs were then soaked in the filtrate and placed onto the agar plates, which were subsequently incubated at 27°C for 48 hours.

For ethanol extraction, 10g of powdered Coptis rhizome were mixed with 40 mL of 70% ethanol and stirred for 2 hours at room temperature (25°C) before being filtered. Blank discs were soaked in the filtrate and placed onto the prepared agar plates, which were then incubated at 27°C for 48 hours.[13]

Lastly, for enzyme-assisted extraction, 10g of powdered Coptis rhizome were mixed with 50 mL of acetate buffer, and the pH was adjusted to 5.5 before 0.3g of cellulase (3% of cellulase w/w) was added and stirred until evenly distributed. Then, 70% methanol was added to the solution.[14] The mixture was subsequently incubated in a water bath at 55°C for 1 hour and centrifuged at 4000 rpm for 20 minutes. Blank discs were then soaked in the supernatant and placed onto the agar plate. All plates were incubated at 27°C for 48 hours.

Zone of Inhibition Test

To measure the antibacterial properties of the extracts, the 'Zone of Inhibition' (ZOI) test also known as the Kirby-Bauer test, was used. This qualitative assay evaluates the susceptibility or resistance of pathogenic bacteria to antibacterial agents.[15] In this procedure, *M. luteus* was spread onto agar plates, and discs soaked in Coptis rhizome were placed on the agar surface. The plates were then incubated at 27°C for 48 hours. Following incubation, the diameters of the zones of inhibition were measured using a vernier caliper to determine the antibacterial activity of each sample.

Ethanol Properties and Control

Ethanol is known to possess antibacterial properties and is commonly used for disinfection and sterilization on surfaces.[6] Therefore, to ensure an accurate measurement of the antibacterial activity of the Coptis rhizome extract, a solvent control subtraction method was used. The 10 agar plates containing ethanol-extracted samples were divided into four sections, with one section designated for the ethanol control. During the data analysis, the ZOI measurements obtained from ethanol-extracted Coptis rhizome discs were subtracted from those of the ethanol control discs.

Data Collection and Statistical Analysis

Results were obtained by measuring the zone of inhibition and were used to calculate the antibacterial efficiency difference between each method. Measurements were taken using a vernier caliper to determine the largest diameter of each zone of inhibition surrounding the discs, including the discs themselves. Then, the measurements from all the discs within each method were averaged to find the average zone of inhibition value for that method. To test the significance of the differences between the extraction methods (aqueous extraction, ethanol extraction, and EAE), one-way analysis of variance (ANOVA) was performed at a significance level of $p < 0.05$.[14] This was followed by Tukey's Honest Significant Difference (HSD) post-hoc test to find the specific pairs of differences.[16]

III. RESULTS AND DISCUSSION

An example of the results observed using the aqueous extraction method is shown in figure 1. After 48 hours, the measured zone of inhibition in all sample discs had a mean of 16.4 mm, which was relatively large compared to the zones in the other two methods. On the other hand, control discs in all trials were completely covered in *M. luteus,* showing no signs of inhibitory effect.

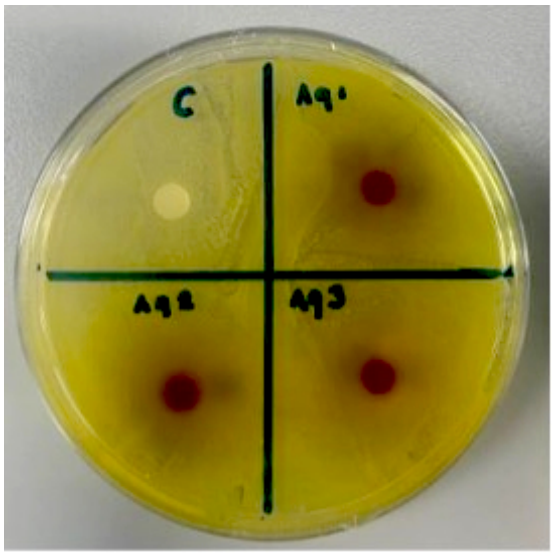

Figure 1. Aqueous extraction assay. This plate shows four different discs, with 'C' being the distilled water control and 'Aq1', 'Aq2', and 'Aq3' being the aqueous-extracted samples. Areas of inhibited bacterial growth are shown around the discs of the aqueous samples.

An example of the results observed using the ethanol extraction method is shown in figure 2. After 48 hours, the measured zone of inhibition in all sample discs was around 14.7 mm, which was slightly lower than in aqueous extraction. The ethanol control discs also formed a zone of inhibition with a mean of 11.35 mm. To determine the antibacterial effectiveness of this extraction method excluding ethanol's intrinsic antibacterial properties, the average zone of inhibition value of the ethanol control discs was subtracted from the average zone of inhibition value of the ethanol-treated samples. Because the measurements included the 6 mm diameter of the discs in all methods, 6 mm was added back to the final

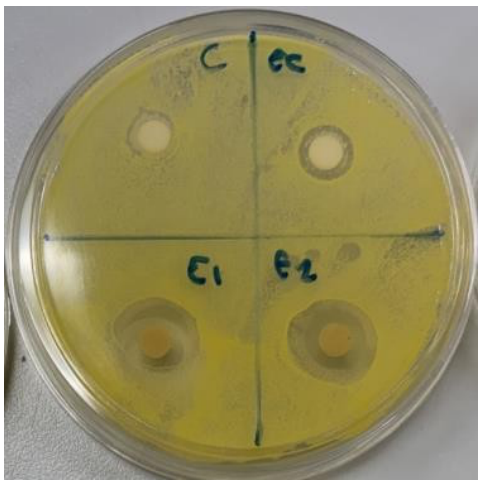

Figure 2. Ethanol extraction assay. This plate consisted of four different discs labeled "C', "Ec", "E1", and "E2". "C" stands for control, which is distilled water. "Ec" is ethanol control, which is the disc soaked in 70% ethanol. "E1" and "E2" are samples of Coptis rhizome extraction from this assay.

calculation. Thus, the zone of inhibition value of the ethanol extraction method was 9.35 mm. In contrast, distilled water discs in all trials were completely covered in *M. luteus*, showing no signs of inhibitory effect.

An example of the results observed using the EAE method is shown in figure 3. After 48 hours, the measured zone of inhibition in some discs has a mean of 10.83 mm due to errors that some discs have zones that could not be measured because they are extremely blurry, causing it to be hard to define between the bacterial growth and the inhibition zone. It is possible that methanol used during the extraction may cause cellulase enzyme denaturation. This affects antibacterial activity by reducing the enzyme's ability to break down Coptis rhizome's cell wall to release its intracellular secondary metabolites.[17]

Figure 4 shows that the standard deviation for ethanol overlap with the other two methods. However, the one-way ANOVA test detected a statistically significant difference at $p < 0.05$. A subsequent Tukey's HSD test result indicated that the aqueous extraction group produced significantly larger zones of inhibition than either the ethanol extraction group ($p=0.0003$) or the enzyme-assisted extraction group ($p=0.0001$). No statistically significant difference was observed between the ethanol and enzyme-assisted extraction groups ($p=0.352$). Thus, the result shows that aqueous extraction has the highest antibacterial activity, since this method appeared to have the largest average zone of inhibition compared to the other two methods, ethanol extraction and EAE.

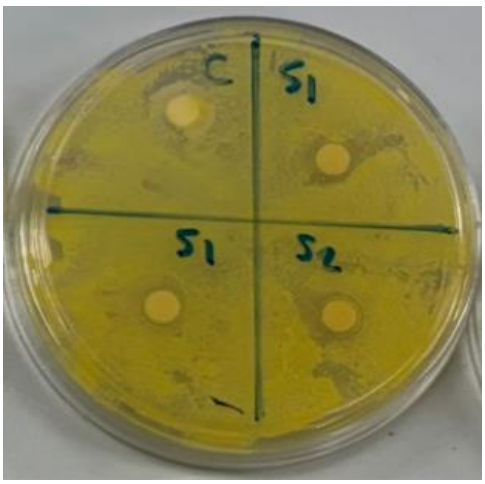

Figure 3. EAE assay. This plate shows four different discs labeled with "C", "S1", "S2", and "S3". "C" represents distilled water control, while "S1", "S2", and "S3" stand for samples of Coptis rhizome extraction from this assay.

While standard protocols were used, it is important to note that this study did not include detailed chemical characterization of the Coptis rhizome extracts. Since the chemical composition of plant extracts can vary depending on factors like plant source and extraction conditions, the antibacterial activity observed in this study may differ from results reported in other findings. The lack of information regarding plant authentication, geographical origin, harvesting conditions, and post-harvest processing introduces potential variability in the chemical composition of the plant material. Such variability may impact the extraction efficiency and antibacterial activity observed, limiting the reproducibility and comparability of the results with other similar studies. As a result, the findings should be interpreted as comparisons between extraction methods rather than exact measurements of individual compound activity.

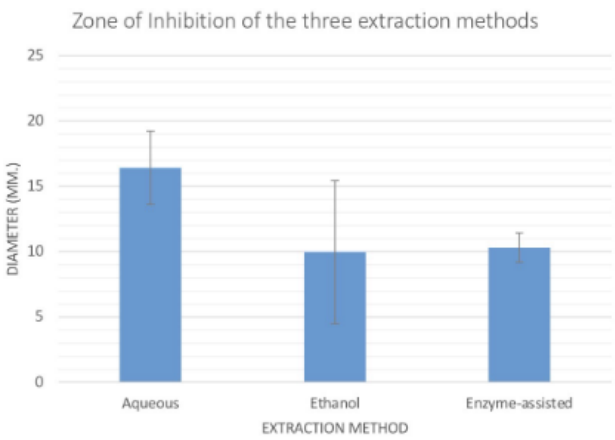

Figure 4. The mean of the zone of inhibition of the three different extraction methods with individual standard deviation bars for each method.

IV. CONCLUSION

This study compared three extraction methods for Coptis rhizome to determine the most efficient method. It is shown that aqueous extract samples were the most effective. Aqueous extracted samples resulted in the largest zone of inhibition, indicating the highest antibacterial activity of Coptis rhizome. In contrast, the ethanol extract exhibited the smallest corrected zone of inhibition. This makes ethanol extraction method to be the least effective extraction method compared to the other methods.

Based on the one-way ANOVA analysis and the post-hoc test, the aqueous extraction group produced the largest zones of inhibition compared to both the ethanol extraction group and the enzyme-assisted extraction group. Additionally, the ANOVA test has also shown our results to be statistically significant ($p < 0.05$).

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