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ANTIBACTERIAL ACTIVITIES OF *CURCULIGO ORCHIOIDES* EXTRACT

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ABSTRACT

Curculigo orchioides has been used for a long period without carrying the complete study about the biological effects on the antibacterial actvity. The motive of this thesis is to contribute for further understanding about *Curculigo orchioide*s and its biological components, especially on the antibacterial activity of its extract. All the tests were performed *in-vitro* to strictly control the conditions. The *Curculigo orchioides* roots were collected from the container and extracted by using decoction method with 40% methanol as the solvent. The antibacterial activity test of the extraction was conducted by agar disk diffusion method while using *Klebsiella pneumoniae* (*K. pneumoniae)* ATCC 13883 and *Pseudomonas aeruginosa (P. aeruginosa)* ATCC 27853. The result indicated that the extraction of *C. orchioides* had the antibacterial activity on on *K. pneumoniae* (17.50 ± 0.50 and 14.17 ± 0.50 mm) and *P. aeruginosa* (18.83 ± 1.89 and 13.27 ± 0.29 mm) with the concentration 500 mg/mL and 100mg/mL respectively. *C. orchioides* and its extraction is a promising source of antibacterial agent The detection of the known and various unknown compounds in the extract also gives promising direction for further study in the pharmaceutical field of this plant.

KEYWORDS: *C. orchioides*, decoction, antibacterial activity, agar disk diffusion.

INTRODUCTION

Curculigo orchioides Gaertn. (*C. orchioides*) commonly known as Kali musli or Sam cau, is a threatened medicinal plant belonging to the family Hypoxidaceae. Its rhizomes have long been used in traditional medicine because of their antiasthmatic, estrogenic, anti-osteoporotic, protective effects against cisplatin-induced cellular damage, antidiabetic, anticancer, and antioxidant properties.[1-7] Although native to India, this species is also widely used in traditional medicine in China and Vietnam and is distributed throughout tropical and subtropical regions of Asia.[8-10] The plant is characterized by palm-like leaves, yellow flowers, black seeds, and erect, subcylindrical rhizomes with brownish external surfaces.[11] Its tuberous roots and rhizomes have traditionally been used as tonics to support health, vitality, and vigor.[12]

Phytochemical investigations conducted between 2013 and 2021 identified approximately 61 compounds from plants of the genus *Curculigo*, with phenolic compounds representing an important group of bioactive constituents.[13] Twenty phenolic compounds were reported from *C. orchioides*, including orcinoside H, orcinol glucosides, curculigosides A, B, C, G, and I, glucosyringic acid, benzyl-O-β-D-glucopyranoside, orcinosides I and J, piperoside, *p*-hydroxycinnamic acid, 3,5-dihydroxy-4-methoxybenzoic acid, and several related phenolic glycosides and derivatives.[13] Among them, orcinoside H, curculigoside I, orcinosides I and J, *p*-hydroxycinnamic acid, and 3,5-dihydroxy-4-methoxybenzoic acid were reported as newly identified compounds. Certain phenolic constituents demonstrated weak anti-osteoporotic activity, an absence of detectable hepatotoxicity toward the human hepatic cell line HL-7702, or xanthine oxidase-inhibitory activity.[14-16]

In addition to these properties, extracts and isolated constituents of *C. orchioides* have been associated with antidiabetic, nephroprotective, anti-osteoporotic, antitumor, antibacterial, anti-inflammatory, antioxidant, neuroprotective, reproductive, and cardioprotective activities.[17–50] These findings indicate that *C. orchioides* is a promising source of structurally and biologically diverse natural compounds. However, the biological effects of its extracts require further experimental investigation.

Despite the development of numerous antibacterial agents, infectious diseases remain a major global health concern because of the emergence and spread of antibacterial-resistant microorganisms.[51] Medicinal plants produce secondary metabolites that contribute to their natural defense against microbial pathogens and may provide potential candidates for

antibacterial development. Previous studies reported that root extracts and flavonoid-containing fractions of *C. orchioides* exhibited antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella Typhi*, and *Klebsiella pneumoniae*.[33,34] However, subsequent evidence confirming these effects remains limited. Therefore, the present study aimed to characterize the phytochemical constituents of *C. orchioides*, with particular attention to their phenolic compounds, and to evaluate the antibacterial activity of its extracts against *P. aeruginosa* and *K. pneumoniae*.

MATERIALS AND METHODS

Materials and Bacterial Strains

Fresh *C. orchioides* roots were procured, cleaned, and sectioned into 3-4 cm × 2–3 cm dimensions to accommodate a round-bottom flask for extraction. All experimental procedures utilized standard laboratory equipment, including a centrifuge, spectrometer, UV transilluminator, buret/chromatography columns, biological safety cabinet, and vortex mixer, alongside standard consumables. Antibacterial assays were performed using the following reference bacterial strains: *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC 13883 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853.

Extraction of the *C. orchioides* roots

The extraction was collected by using decoction method using 70% of methanol as the extraction solvent. First, 200 g of *C. orchioides* roots was cut into small pieces fitting the round-bottom flask. The flask was then filled with 200 mL of methanol 70%, the mixture was boiled in distilled system to remove essential oils. The extract was used for antibacterial activity test. Before using the extraction for any testing, the extract was centrifuged to get rid of the residue. The obtained extract was then evaporated to eliminate the methanol. The residue was dissolved in distilled water and called Crude extract.

Antibacterial activity

In the study, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 27853 were observed under microscope after staining gram to check the morphology. They were grown in LB broth overnight at 37 ^{o}C in the incubator. The stock was then used to spread on a clean agar petri dish to collect single colony stored in a LB broth.

The test was carried out by using agar disk diffusion method.[52] The agar petri plates were prepared before the test at least 12 hours to make sure there was no foreign pathogens. An

appropriate amount of bacteria broth stock after vortexing (20-50 µL depending on the size of the colony) was spread on all over the surface of the agar dish. Then put the disks with the positive, negative and extraction in 3 equal parts of the agar dish. The positive control was 70% ethanol, negative control was distilled water, and the sample test was the extract after eliminating the ethanol (the test was performed at two identical concentrations 500 mg/mL and 100 mg/mL of the extraction, under 100 mg/mL the extraction did not show any effect, but the data is not shown in this test). The agar plate was finally incubated at room temperature for overnight in the incubator. The inhibition zones surrounding of the disk were measured to check the sensitivity of the pathogenic bacteria to the extract. The experiments were repeated to ensure the results.

RESULTS AND DISCUSSION

Antibacterial activity

In both cases, the extraction exhibited different level of inhibition on the bacteria strains *K. pneumoniae* ATCC 13883 and *Pseudomonas aeruginosa (P. aeruginosa)* ATCC 27853. Results were showed in the table 1 and 2.

Table 1: Zones of inhibition (mm) of distilled water, ethanol 70%, *C. orchioides* extraction at 500 mg/mL.

Tested microorganisms	Zone of inhibition (mm)		
	Distilled water	Ethanol 70%	*C. orchioides* extraction
K. pneumoniae	NA	14.32 ±0.29	17.50±0.50
P. aeruginosa	NA	15.17±0.76	18.83±1.89

(NA: No activity)

Table 2: Zones of inhibition (mm) of distilled water, ethanol 70%, *C. orchioides* extraction at 100 mg/mL.

Tested microorganisms	Zone of inhibition (mm)		
	Distilled water	Ethanol 70%	*C. orchioides extraction*
K. pneumoniae	NA	13.85 ± 0.46	14.17 ± 0.50
P. aeruginosa	NA	14.92 ± 0.35	13.27 ± 0.29

(NA: No activity)

More specifically, the extract had the impact on *K. pneumoniae* (17.50 ± 0.50 and 14.17 ± 0.50 mm) and *P. aeruginosa* (18.83 ± 1.89 and 13.27 ± 0.29 mm). It also stated that the 500 mg/mL extract overall have a larger zone of inhibition compared to the 100 mg/mL one.

By using statistic test to compare the significant difference between the inhibition zone of ethanol 70% in table 3 and inhibition zone of ethanol 70% in table 4. It was supposed that there was no difference between them, since the p-value is greater than 0.05, fail to reject the null hypothesis. By using statistical test to compare the significant difference between the difference concentration inhibition zone, suppose there is no significant difference between them. Since the p-value is smaller than 0.05, reject the null hypothesis. Which mean the concentration effects the inhibition zone. In this assay, the 500mg/mL concentration of the extract had larger significant zone of inhibitor.

And also, by comparing the inhibition zone between the ethanol 70% and the extraction at both concentrations, the number always says that the inhibition zone between them is significantly different (in both cases, the extraction inhibition zone was bigger). Therefore, at both concentration 100mg/mL or 500mg/mL, the extraction always had better effect compared to ethanol 70% in the antibacterial activities. The antibacterial agents were being exploited based on the study results.

CONCLUSION

The extraction of *C. orchioides* exhibited antibacterial activity with different level on different types of bacteria including *K. pneumoniae* ATCC 13883 and *P. aeruginosa* ATCC 27853. That information will contribute to pharmaceutical development in the near future and provide more data for further *C. orchioides* study or maximize its potential in many other fields.

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