Research Article

Antibacterial activity of *Murdannia loriformis* (Hassk.) isolated from the surface of instruments used in kindergarten classrooms

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Abstract

In traditional medicine, *Murdannia loriformis* (Hassk.) has been used to treat a wide range of illnesses, such as chronic bronchitis, early-stage malignancies, colds, throat infections, pneumonia, the flu, and wound healing. This study aimed to examine the antibacterial activity of *M. loriformis* leaf ethanol extract. In this study, 72 samples from the surface of instruments used in kindergarten classrooms, such as student desks, wooden toys, and plastic toys, were collected and isolated as types of bacteria, i.e., *S. aureus*, *S. epidermidis*, and *E. coli*. All samples were contaminated with *S. aureus* (70.8%) and *S. epidermidis* (62.5%). However, *E. coli* was not contaminated by the samples. The results of antibacterial activities from *M. loriformis* leaf ethanol extract at concentrations of 10, 20, and 30 mg/mL using the agar well diffusion method revealed that all concentrations of *M. loriformis* leaf ethanol extract could inhibit *S. aureus* with a mean diameter of inhibitory effects at 6.5 ± 0.50, 15.17 ± 0.76, and 17.50 ± 0.50 mm, respectively. Moreover, the results showed that the ethanol extract of *M. loriformis* leaves had a minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against *S. aureus*, with MIC values of 6.25 mg/mL and MBC values of 100 mg/mL. Therefore, this study demonstrated *M. loriformis* leaf ethanol extract as an antibacterial agent against *S. aureus* infections found in kindergarten classrooms.

Keywords: Antibacterial activity, Kindergarten classrooms, *Murdannia loriformis* (Hassk.)

Introduction

Humans can develop a variety of clinical symptoms, including skin and soft tissue infections, abscesses, pneumonia, and even bloodstream infections, due to the commensal and pathogenic bacterium *Staphylococcus aureus* [1]. In a previous study, it was reported that a high proportion of *S. aureus* nasal colonization in schoolchildren was found, and *S. aureus* bacteria were found on classroom floors and classroom desks [2]. Moreover, studies have revealed that *S. aureus* is a common colonizer of children's skin and mucus membranes, with the potential to cause serious harm [3]. Young children tend to put their hands and objects in their mouths, and they are unable to maintain their own hygiene. Children in kindergartens are typically confined to a small amount of space, which may play a role in the spread of potential pathogens through close contact and toy sharing [4]. Furthermore, staphylococcal invasive infections led to musculoskeletal infections in children [5]. Furthermore, the study of bacterial contamination of children's toys in rural daycare centers and households in South Africa reported that 61 (24.79%) toys from 246 samples were contaminated with *E. coli* [6]. In the prevalence of bacteria isolated in samples from public and private schools, it was found that bacterial strains isolated from the two schools had a mean percentage of 37.1% *Staphylococcus* sp. and 4.6% *E. coli* [7].

In Thailand, people with diabetes and cancer frequently take Beijing grass, also known as *M. loriformis* [8-9]. In traditional Thai medicine, *M. loriformis* is used traditionally for self-treatment by cancer patients among rural communities. According to several cancer patients, consuming fresh drinks of *M. loriformis* could prolong life and reduce the side effects of chemotherapy and radiation. [10-11]. A literature review on the antibacterial activity of herbal extracts found that *M. loriformis* has antibacterial properties, *Propionibacterium acnes* and *Staphylococcus epidermidis*, which can grow on human skin [12]. A MIC value of 1.25 mg/mL was discovered to be effective for *M. loriformis* against *S. epidermidis* in previous research and the MBC value was >5 mg/mL. [12]. The phytochemical investigation of *M. loriformis* includes phytosterylglycoside and glycosphingolipid, which have immunomodulatory effects and moderate cytotoxic activity against breast and colon cancer cell lines [13].

Despite available data, knowledge concerning the prevention of *S. aureus* infection in schoolchildren by applying natural products is still unknown. Therefore, the present study aimed to isolate *S. aureus* from the surfaces of instruments in kindergarten classrooms and to determine the effect of extracts from *M. loriformis* leaf ethanol extract against pathogenic bacteria. The data obtained from this research will be useful in determining the prevention and control of such bacterial diseases among preschool children and providing information for the development of *M. loriformis* products for use in the inhibition of further growth of pathogenic bacteria.

Materials and methods

Chemicals and reagents

Culture media such as Mannitol Salt Agar, Brilliant Green Lactose Bile Broth, EC broth, Muller Hilton Agar, and Muller Hilton Broth were obtained from Himedia (India). Analytical reagent grades of dimethyl sulfoxide (DMSO) and ethanol were purchased from Merck KGaA (USA). The antibiotic tetracycline was provided by Sigma-Aldrich (USA).

Plant material

Murdannia loriformis (Hassk.) leaves were collected from Ban Dong Bang Samunprai Community, Tambon Dong Khi Lek, Mueang District, Prachin Buri Province, Thailand. After being cleaned with water, the samples were sliced into small pieces and dried at 45 °C. After being crushed, the dry plant samples were kept in a sealed container.

Plant extraction

Plant extraction was performed by maceration with 95% ethanol (1000 ml.) and 150 grams of *M. loriformis* leaf powder under a tightly closed container and left at 25 °C for 5 days, followed by filtration through a thin white cloth. The solvent of each filtrate was evaporated at 40 °C under reduced pressure at 175 mbar using a rotary vacuum evaporator. The following formula was used to get the yield percentages: Weight of dry extract / weight of dry sample × 100 = extract yield (%) [14].

Sample Collection

Samples were randomly collected from the surfaces of classroom instruments, including student desks (n = 24), wooden toys (n = 24), and plastic toys (n = 24) in kindergarten classrooms. The sample included 8 classrooms from kindergarten school, which is 3-6 years of students. A sterile cotton swab was dipped in a test tube containing phosphate buffered saline (PBS) and rubbed against the surface area of the classroom instrument, after which the cotton swab was dipped into the tube and broken to be able to fit the tube. The cap was closed, and the buffer tube was shaken with a vortex mixer.

Microbiological Detection of Staphylococci

Samples were serially diluted with PBS at 10^{-1}, 10^{-2}, and 10^{-3}. Each dilution level was transferred 0.1 mL into Petri dishes containing Mannitol Salt Agar (MSA), and the sample was spread over the media surface. The process was repeated three times each. Incubation at 35 °C was carried out for 48 hours. *S. aureus* was identified by selected convex colonies with smooth yellow edges. *S. epidermidis* colonies did not change the color of the MSA medium, which was confirmed by gram dyeing. Subsequently, *S. aureus* was preserved in nutrient agar slant tubes at 4 °C until use [15].

Microbiological Detection of *E. coli*

The Most Probable Number (MPN) method was used for the analysis of coliforms, fecal coliforms, and *E. coli* according to the method recommended by the U.S. Food and Drug Administration [16]. The MPN method consisted of three steps: a presumptive test, a confirmed test, and a complete test.

Presumptive test

Samples were serially diluted with PBS at 10^{-1}, 10^{-2}, and 10^{-3}. Each dilution level was transferred 0.1 mL into LST tubes for a 3-tube MPN analysis. LST tubes were kept at 35°C, and the agarose reaction tube was recorded at 24 h, followed by displacement of the medium in a fermentation vial or effervescence when tubes were gently agitated. Negative tubes were incubated for an additional 24 hours, and reactions were recorded again. Following that, positive (gas) tubes were used to validate the test.

Confirmed test for coliforms

A loopful of suspension was transferred from each positive gassing LST to a brilliant green lactose bile broth (BGLB) tube. After 48 hours of incubation at 35°C, BGLB tubes were checked for gas generation. In order to determine the most likely number (MPN) of coliforms, the percentage of confirmed gassing LST tubes for three consecutive dilutions was used.

Confirmed test for fecal coliforms and *E. coli*

An EC broth tube was filled with a loopful of each suspension from each gassing BGLB tube. The EC tubes were then incubated for 24 hours at 44.5°C and checked for gas generation. Reactions were once again observed after a further 24 hours of incubation in negative tubes. The fecal coliform MPN was computed using the test results.

Completed test for *E. coli*

Each gaseous EC tube was loop-full and streaked onto an Eosin Methylene Blue (EMB) agar plate for isolation. The plate was then incubated at 35°C for 18–24 hours. The dark-centered, flat, metallic-looking *E. coli* colonies on the plates were checked for suspicious ones.

Agar well diffusion method

The antibacterial activities of *M. loriformis* leaf ethanol extract against *S. aureus* were tested with the agar well diffusion method, as described by CLSI (Clinical and Laboratory Standards Institute Guidelines) [17]. *S. aureus*, obtained by surface isolation of the classroom instrument, was cultured in a nutrient broth medium for 12–16 hours at 37 °C. Following that, sterile normal saline was used to make suspensions of the bacterial isolates, which were then corrected to 0.5 McFarland's standard solution. A sterile cotton swab was dipped into an inoculated test tube and spread on the nutrient agar by a three-dimensional swab method. The agar wells were prepared by using a sterilized cork borer with a 6 mm diameter. Three different concentrations of the *M. loriformis* leaf ethanol extract solutions (10, 20, and 30 mg/mL of *M. loriformis* leaf ethanol extract) were mixed with 10% DMSO and then added to the agar well. DMSO was used as a negative control, while 2 µg/mL of tetracycline was used as a positive control. The dishes were incubated at 37 °C for 24 h. The antibacterial activity of *M. loriformis* leaf ethanol extract was determined by measuring the inhibition zone diameter in millimeters. Each trial was performed in triplicate [18].

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The MIC and MBC of *M. loriformis* leaf ethanol extract against *S. aureus* were determined using a broth microdilution method. The antibiotic used as a reference was tetracycline. As the lowest concentration that totally inhibited observable growth, the MIC was defined. Using a sterile loop streaked on new media, the concentration that yielded significant MIC values was determined to be MBC. Every assay was run three times [19].

Results

The percentage of yield from *M. loriformis* leaf ethanol extract was 6.5%, and the color of the extract was dark green. The samples from student desks, wooden toys, and plastic toys were contaminated with *S. aureus* and *S. epidermidis*. However, *E. coli* contamination was not found. In addition, the prevalence of *S. aureus* was found to be 51 samples (70.8%) higher than that of *S. epidermidis*, which was found to be 45 samples (62.5%). The prevalence of *S. aureus* from student desks, wooden toys, and plastic toys was 62.5, 75.0, and 75.0%, and the prevalence of *S. epidermidis* was 62.5, 50.0, and 75.0%, respectively. This result showed that the most contaminated classroom instruments with *S. aureus* and *S. epidermidis* were plastic toys (Table 1).

The antibacterial activity was performed against *S. aureus* and *S. epidermidis* by the agar-well diffusion method. Tetracycline was used as a positive control, and DMSO was used as a negative control. Tetracycline was presented in the inhibition zone at 25.17 mm. *M. loriformis* leaf ethanol extract was used to vary concentrations at 10, 20, and 30 mg/ml. The result shown that DMSO was not found to inhibit *S. aureus* and *S. epidermidis. S. epidermidis* does not have antibacterial activities from *M. loriformis*. In addition, the results showed that the extract had the potential to inhibit *S. aureus*, with mean inhibitory activity diameters ranging from 6.50, 15.17, and 17.50 mm (inhibition zone). All concentrations of the extract were able to inhibit bacterial growth significantly differently (p-value <0.000) (Table 2).

Determination of antibacterial activity *M. loriformis* leaf ethanol extract was tested at minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The extract was used at concentrations of 0.047–100 mg/mL. Tetracycline was used as a positive control with concentrations of 0.047–100 mg/mL. It was found that the MIC and MBC of tetracycline were 3.12 and 6.25 mg/mL, respectively. The result exhibited that *M. loriformis* leaf ethanol extract can inhibit *S. aureus*; the MIC value of the extract was 6.25 mg/mL. Furthermore, the extract had bactericidal activity, as determined by the MBC test. The MBC value of the extract was 100 mg/mL (Table 3). Although, a literature review showed that antibacterial activity from whole *M. loriformis* ethanol extraction by disc diffusion method was not shown for *S. aureus* antibacterial activity at 0.0005–5 mg/mL [20].

Table 1 Prevalence of bacterial contamination of kindergarten classroom instruments

Bacterial strains	Kindergarten classroom instruments (n=72)			
	Student desks (n=24)	Wooden toys (n=24)	Plastic toys (n=24)	Total (%)
S. aureus	15 (62.5)	18 (75.0)	18 (75.0)	51 (70.8)
S. epidermidis	15 (62.5)	12 (50.0)	18 (75.0)	45 (62.5)
E. coli	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 2 Antibacterial activity of *M. loriformis* leaf ethanol extract against *S. aureus*

Concentration (mg/mL)	Inhibition zones ± SD (mm)
M. loriformis leaves ethanol extract 10 mg/mL	6.50 ± 0.50
M. loriformis leaves ethanol extract 20 mg/mL	15.17 ± 0.76
M. loriformis leaves ethanol extract 30 mg/mL	17.50 ± 0.50
DMSO	6.00 ± 0.00
Tetracycline 2 mg/mL	25.17 ± 0.76

p-value <0.000

Table 3 Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) of *M. loriformis* leaf ethanol extract and tetracycline

Bacterial strains	MIC (mg/mL)		MBC (mg/mL)	
	Extract	Tetracycline	Extract	Tetracycline
S. aureus	6.25	3.12	100	6.25

Discussion

From this study, it was found that student desks, wooden toys, and plastic toys were contaminated with *S. aureus* and *S. epidermis*. Consistent with Ali, Al-Harbi, and Rahman [21], 25 samples of toys collected from 2 kindergartens and 1 child care center were contaminated with *S. aureus* and *S. epidermis*. The result of this research confirmed that toys serve as media for the transmission of diseases. In kindergarten 1, bacteria were isolated from 6 samples of toys contaminated with *S. aureus* at 50%. In kindergarten 2, bacteria isolated from 10 samples showed *S. aureus* at 20%. While the child care center presented *S. aureus* at 55.5%, which is isolated from 9 samples.

In addition, the study's findings showed that the most contamination of classroom instruments with *S. aureus* and *S. epidermidis* were plastic toys. Boretti, Corrêa, Santos, Leão, and Silva [22] found that 87% of *Staphylococcus* spp. were isolated from toys, with plastic toys being the most contaminated, which is similar to the results obtained from this study. Moreover, toys for kids in hospital play areas, including cardboard, plastic, wooden, plush, rubber, and metal toys, may contain harmful microorganisms. [23]. *S. aureus* was isolated from toys provided to children at a children's hospital. It was concluded that toys could be contaminated with pathogenic bacteria and pose unnecessary risks for nosocomial infection [24].

E. coli bacteria can be found in food, the environment, and the intestines of both humans and animals. Therefore, the presence of *E. coli* in the environment suggests that there may have been fecal contamination and the presence of additional fecal microbes, including diseases [6]. From our research, *E. coli* was not found on equipment in kindergarten classrooms due to the kindergarten children' good hand hygiene, particularly their practice of washing their hands after using the toilets.

However, Kasorn & Nathapindhu [25] reported that *E. coli* contaminated the surfaces of instruments used, including student tables, faucet washbasins, faucet drinks, wooden toys, and plastic toys, in 11 kindergartens in Khon Kaen municipality.

This result from our study revealed that *M. loriformis* has antibacterial activity. A previous study reported that *M. loriformis* ethanol extract did not have antibacterial activity [20]. This study aimed to investigate the minimum concentration (MIC) and minimum bactericidal concentration (MBC) of *M. loriformis* leaf ethanol extract against *S. aureus.* The study found that the MIC value of *M. loriformis* leaf ethanol extract inhibited *S. aureus* at a concentration of 6.25 mg/mL. Moreover, the bactericidal activity of *M. loriformis* leaf ethanol extract for *S. aureus* was 100 mg/mL. Phytochemical analysis of *M. loriformis* extract revealed that it is composed of phenolics, flavonoids, condensed tannins, chlorophylls, alkaloids, and steroids [26]. Phenolic chemicals can disrupt cellular membrane permeability, inhibit virulence factors such as enzymes and toxins, and prevent the formation of bacterial biofilms [27]. Furthermore, flavonoids can destroy the permeability system of substances within the cells of microorganisms, causing the cells to be unable to do normal activities and thus affecting the growth of microorganisms [28].

Conclusions

Classroom equipment provided to children in kindergarten classrooms poses a risk of bacterial infection and has the capacity to cause severe illnesses. Child caregivers should be more concerned about this significant issue. Monitoring drug-resistant bacterial contaminants in children's belongings is an important and necessary task in order to combat the life-threatening infections caused by such microbial pathogens.

From the results, *M. loriformis* extract exhibited activity against *S. aureus.* Thus, *M. loriformis* extraction can be applied for infection prevention. For example, it can be used as an ingredient in antimicrobial products to reduce the number of pathogenic bacteria. It also promotes the conservation of Thai medicinal plants and encourages the use of Thai medicinal plants for more benefits.

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