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Research Article
A New Potential Source of Anti-pathogenic Bacterial Substances from *Zamioculcas zamiifolia* (Lodd.) Engl. Extracts

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Abstract

Background and Objective: The increase of antibiotic-resistant bacteria is a problem for global health that needs to find new antibiotic drugs. The plant is the potential source of antibiotic substances that important to solve the antibiotic-resistant bacteria. This study was aimed to evaluate the antibacterial activity of *Zamioculcas zamiifolia* stem extracts against nine human pathogenic bacteria. **Materials and Methods:** *Z. zamiifolia* stems were extracted with five extraction solvents. The screening of antibacterial activity of stem extract was measured using agar disc diffusion assay. The Minimal Inhibition Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values of extracts were determined using the broth microdilution assay and colorimetric assay. **Results:** The results indicated that the lowest MIC value of 0.09 mg mL^{-1} against *Staphylococcus aureus* TISTR 1466 was obtained from hexane extraction. The lowest MBCs value of 1.56 mg mL^{-1} against *Bacillus cereus* TISTR 2373, *Listeria* spp. and *Escherichia coli* TISTR 527 were obtained from ethanol and methanol extractions. **Conclusion:** The ethanolic and methanolic stem extracts of *Z. zamiifolia* demonstrated the highest anti-human pathogenic bacterial activity. This is the first report to demonstrate the high potential of antibacterial substance from *Z. zamiifolia* stem extracts, which can be developed further as a natural drug for treating bacterial infectious diseases.

Key words: Anti-bacterial activity, human pathogenic bacteria, *Zamioculcas zamiifolia* (Lodd.) Engl., ZZ plant, stem extracts, natural drug, plant extraction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Misuses of antibiotics in humans and animals lead to antibiotic resistance in bacteria. The novel, effective and affordable antibiotic drugs to treat infectious diseases are the most major challenge in global health care[1]. Plants constitute a good source of antibiotic compounds, in regards to the variety and diversity of their chemical structures[2] and also found in marine organisms[3]. Many plants were reported that contained antibacterial activity such as *Albizia adianthifolia*, *Alchornea laxiflora*, *Laportea ovalifolia*[2], *Allium sativum*, *Bunium persicum*[4], *Canarium schweinfurthii*[5], *Rosmarinus officinalis* L.[6], *Rhizophora mucronata*, *Rhizophora apiculata*, *Rhizophora annamalayana*[7], *Chimonanthus salicifolius*[8], *Carum copticum* L.[9], *Thymus capitatus*[10], *Uapaca heudelotti*[11], *Murraya koenigii*[12], *Teucrium polium* L.[13], *Cremaspora trifloral*, *Hypericum roeperianum*[1], *Scutellaria baicalnsis*[14] and *Spathiphyllum wallisii*[15].

An unusual drought-tolerant plant *Zamioculcas zamiifolia* (Lodd.) is a native drought-tolerant medicinal plant which found in tropical East Africa and subtropical southeast Africa[16]. This forest plant develops short sprouts from a thick underground tuber-like rhizome to reserve water for its survival without water for longer periods[17]. It is an ornamental potted foliage plant in the family Araceae, which a high potential to reduce the concentration of many contaminants indoor air including Benzene, Toluene, Ethylbenzene and Xylene (BTEX), respectively[18]. The novel natural products main compound of the *Z. zamiifolia* leaves, apigenin 6-C-(6"-(3-hydroxy-3-methyl-glutaroyl)-β-glucopyranoside was reported recently[16]. However, the antibacterial activity of *Z. zamiifolia* has yet been evaluated.

This study aimed to evaluate the antibacterial activity of *Z. zamiifolia* extracts against nine pathogenic bacteria including *Bacillus subtilis* TISTR 008, *Escherichia coli* TISTR 527, *Staphylococcus aureus* TISTR 1466, *Bacillus cereus* TISTR 2373, *Staphylococcus epidermidis* TISTR 518, *Pseudomonas aeruginosa* TISTR 2370, *Klebsiella pneumoniae* subsp. *pneumoniae* TISTR 1383, *Staphylococcus aureus* PK and *Listeria* spp. This is the first report that the antibacterial activity of *Z. zamiifolia* extracts was demonstrated *in vitro* which benefits for antibiotic drug development.

MATERIALS AND METHODS

Study area: All the experiments were performed during October, 2019-April, 2020 in the Microbiology Laboratory, Major of General Science, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand.

Chemicals and reagents: Hexane, Dichloromethane, Ethyl acetate, Ethanol and Methanol were purchased from QRëC™ (Republic of New Zealand), Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich Co. (St. Louis, Missouri, U.S.A.), Mueller Hinton Broth (MHB), Nutrient Broth (NB) and Bacterial Agar were purchased from HiMedia (HiMedia Laboratories Pvt. Ltd, India).

Human pathogenic bacteria: A total of 9 human pathogenic bacterial strains were used in this research: *Bacillus subtilis* TISTR 008 (*B. subtilis* TISTR 008), *Escherichia coli* TISTR 527 (*E. coli* TISTR 527), *Staphylococcus aureus* TISTR 1466 (*S. aureus* TISTR 1466), *Bacillus cereus* TISTR 2373 (*B. cereus* TISTR 2373), *Staphylococcus epidermidis* TISTR 518 (*S. epidermidis* TISTR 518), *Pseudomonas aeruginosa* TISTR 2370 (*P. aeruginosa* TISTR 2370), *Klebsiella pneumoniae* subsp. *pneumoniae* TISTR 1383 (*K. pneumoniae* TISTR 1383) were obtained from the Thailand Institute of Scientific and Technological Research culture collection (TISTR culture collection), Thailand. *Staphylococcus aureus* PK (*S. aureus* PK) and *Listeria* spp. were obtained from Major of Microbiology, Faculty of Science, Mahasarakham University, Thailand. All human pathogenic bacteria were cultured using Nutrient Broth (NB) before use.

Plant sample collection and preparation of extracts: *Z. zamiifolia* stem was collected from Ban Tha Muang, Selaphum, Roi Et, Thailand. The collected stems were washed three times with water, cut into small pieces and dried in a hot air oven (POL-EKO-APARATURA company, Wodzisław Śląski, Poland) at 50°C for 48 hrs. The dried stems were ground and biochemical substances were extracted with five extraction solvents including hexane, dichloromethane, ethyl acetate, methanol and ethanol (QRëC™, Republic of New Zealand) at a ratio of 1:10 (w/v). The extractions were carried out on a shaking at room temperature for 24 hrs before filtration. The filtrates were concentrated using a Rotavapor (Buchi, Switzerland) and the percent yield was calculated[15]. The extracts were then diluted with dimethyl sulfoxide (DMSO) to the final concentration at 500 mg mL^{-1}.

$$\text{Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \times 100$$

Screening for antimicrobial activity of *Z. zamiifolia* stem extracts using agar disc diffusion assay: The antagonistic

activity of each stem extract was tested by the disk diffusion method[19]. Overnight bacterial cultures were adjusted the cell concentration at OD_{600}-0.1. One hundred microliters of each pathogenic bacterium were spread onto Mueller Hinton Agar (MHA) and a sterile paper dish (0.6 mm) was placed on MHA. Ten microliters of each extract were dropped onto the center of the paper dish and DMSO and Kanamycin were used as a control. The extracts were allowed to diffuse for 15 min before MHA plates were incubated at 37°C for 24 hrs. The zone of inhibition formation around the paper disks was measured.

***In vitro* antibacterial activity determination using a broth microdilution assay:** The broth microdilution assay was used to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values. *Z. zamiifolia* stem extracts were twofold serial diluted in 96-well plate containing MHB to obtain various concentrations. The overnight bacterial inoculum was adjusted the cell concentration at OD_{600}-0.1 before adding 100 µL into each well. Kanamycin (50 mg mL^{-1}) and DMSO were used as a positive and negative control, respectively. The 96-well plates were incubated at 37°C for 24 hrs. As an indicator of bacterial growth, 50 µL iodonitrotetrazolium chloride (INT) was added in each well of the 96-well plate and was incubated at 37°C for 1 h[15]. The well containing the bacterial growth turned into pink color whereas the well without bacterial growth remained yellow color. The MIC was considered as the lowest concentration of the extract that completely inhibits the bacterial growth. The MBC was considered as the lowest concentration of extract that kills bacteria that did not produce a color change after the addition of INT[5].

Data analysis: In this study, it was used experimental design followed by descriptive analysis.

RESULTS AND DISCUSSION

Extraction yields: The extraction of *Z. zamiifolia* stem was extracted using 5 extraction solvents with shaking for 24 hrs. The highest yield was obtained from ethanolic extract of 10.98% while the least yield was obtained from hexane extraction (Table 1).

Antibacterial activity determination using agar disc diffusion assay: On the initial test, the results indicated that *Z. zamiifolia* stem extracts showed antibacterial activity by suppressing bacterial growth with various degrees, which methanol extract exhibited the largest inhibition zones at 10.0 mm against *Listeria* spp. (Table 2) which similar to previously reported by Srikacha and Ratananikom[20] that ethanol is the most suitable solvent for extraction. Hexane extract inhibited the growth of all gram-positive pathogenic bacteria, whereas dichloromethane extract only showed an effect on *B. subtilis* TISTR 008. Ethyl acetate extract did not have antibacterial activity against any bacteria tested. The result of inhibition zone from this research was.

The results of antibacterial activity assay by disc diffusion suggested that *S. aureus* PK, *K. pneumoniae* TISTR 1383 and *E. coli* TISTR 527 were the most resistant strains to *Z. zamiifolia* stem extracts followed by *Listeria* spp., *P. aeruginosa* TISTR 2370, *S. aureus* TISTR 1466, *B. cereus* TISTR 2373, *S. epidermidis* TISTR 518 while *B. subtilis* TISTR 008 was the most susceptible strain to the extracted plants,

Table 1: Extraction yield of the *Z. zamiifolia* stem extracts

Extraction solvent	Yields (%)
Hexane	0.04
Dichloromethane	0.51
Ethyl acetate	0.87
Methanol	5.17
Ethanol	10.98

Table 2: Diameter of inhibition zones (mm) of *Z. zamiifolia* stem extracts against nine pathogenic bacteria at 500 mg mL^{-1} concentration

	Inhibition zones (mm)								
	Gram-positive pathogenic bacteria						Gram-negative pathogenic bacteria		
Extraction solvent	*B. subtilis* TISTR 008	*S. aureus* TISTR 1466	*B. cereus* TISTR 2373	*S. epidermidis* TISTR 518	*S. aureus* PK	Listeria spp.	*E. coli* TISTR 527	*P. aeruginosa* TISTR 2370	*K. pneumoniae* TISTR 1383
Hexane	9.5	7.5	9.0	7.0	9.0	-	-	7.5	-
Dichloromethane	9.0	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-	-	-
Methanol	6.5	8.0	9.0	7.0	-	10.0	6.0	9.0	-
Ethanol	8.0	7.0	9.0	6.0	-	6.5	-	8.5	8.0
DMSO	-	-	-	-	-	-	-	-	-

-: No antibacterial activity

Table 3: MIC value of *Z. zamiifolia* stem extracts against human pathogenic bacteria (mg mL^{-1})

	Minimum inhibitory concentrations (mg mL^{-1})								
	Gram-positive pathogenic bacteria						Gram-negative pathogenic bacteria		
Extraction solvent	*B. subtilis* TISTR 008	*S. aureus* TISTR 1466	*B. cereus* TISTR 2373	*S. epidermidis* TISTR 518	*S. aureus* PK	Listeria spp.	*E. coli* TISTR 527	*P. aeruginosa* TISTR 2370	*K. pneumoniae* TISTR 1383
Hexane	0.39	0.09	0.39	0.78	0.19	-	-	0.78	-
Dichloromethane	6.2	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-	-	-
Methanol	6.2	12.5	0.78	12.5	-	0.78	0.78	6.2	-
Ethanol	6.2	6.2	0.78	12.5	-	0.78	-	6.2	6.2
Kanamycin	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.039	<0.004
DMSO	-	-	-	-	-	-	-	-	-

-: No antibacterial activity

Table 4: MBC value of *Z. zamiifolia* stem extracts against human pathogenic bacteria (mg mL^{-1})

	Minimal bactericidal concentration (mg mL^{-1})								
	Gram-positive pathogenic bacteria						Gram-negative pathogenic bacteria		
Extraction solvent	*B. subtilis* TISTR 008	*S. aureus* TISTR 1466	*B. cereus* TISTR 2373	*S. epidermidis* TISTR 518	*S. aureus* PK	Listeria spp.	*E. coli* TISTR 527	*P. aeruginosa* TISTR 2370	*K. pneumoniae* TISTR 1383
Hexane	3.12	6.2	12.5	12.5	12.5	-	-	25	-
Dichloromethane	12.5	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-	-	-
Methanol	12.5	25	3.12	25	-	1.56	1.56	12.5	-
Ethanol	12.5	12.5	1.56	25	-	1.56	-	12.5	12.5
Kanamycin	<0.004	<0.004	<0.004	<0.004	>25	0.019	0.019	0.039	0.625
DMSO	-	-	-	-	-	-	-	-	-

(-): no antibacterial activity

respectively. Methanol and ethanol were the most effective solvent to extract antibacterial substances out of *Z. zamiifolia* stems. Thus, follow-up experiments were conducted to determine their MIC and MBC against these bacteria.

***In vitro* antibacterial activity assay:** Antibacterial activity of the extracts was further evaluated by MIC and MBC measurement using broth microdilution assay. The MICs of *Z. zamiifolia* stem extracts were ranged between 0.09-12.5 mg mL^{-1}. Among five solvent extracts, hexane extract showed the highest antibacterial activity against gram-positive pathogenic bacteria, which MICs of *Z. zamiifolia* stem extracts against *S. aureus* TISTR 1466, *S. aureus* PK, *B. subtilis* TISTR 008 and *B. cereus* TISTR 2373 were 0.09, 0.19, 0.39 and 0.39 mg mL^{-1}, respectively (Table 3). Dichloromethane extract was only effective against *B. subtilis* TISTR 008 with MIC value of 6.2 mg mL^{-1}. Ethanol and methanol were the most effective extracts against both gram-positive and gram-negative bacteria. Achika *et al.*[11] recently reported that ethyl acetate fraction of the stem bark of *Uapaca heudelotti* was active against *E. coli*, *B. subtilis*, *K. pneumoniae*, *S. aureus* with MICs value of 3.12-12.5 µg mL^{-1}. Abuga *et al.*[12] also found the ethyl acetate extract of the leaves gave the lowest MIC value against *S. aureus* and *E. coli* 0157:H7 at 15.63 µg mL^{-1}. Trinh *et al.*[21] reported that *A. conyzoides* flower showed the most potent effect on uropathogenic *E. coli* and *K. pneumoniae* with MIC at 1.25-10 and 5-12.5 mg mL^{-1}, respectively.

The MBCs of *Z. zamiifolia* stem extracts against the bacteria were ranged between 1.56-25 mg mL^{-1}. The ethanol and methanol extract demonstrated the highest antibacterial activity against *B. cereus* TISTR 2373, *Listeria* spp. and *E. coli* TISTR 527 with MBCs value of 1.56 mg mL^{-1} (Table 4). The MIC and MBC value of ethanol extracts of *Z. zamiifolia* stem were similar to previously reported by Dzotam *et al.*[5] that ethanol extract of *Canarium schweinfurthii* against *E. coli* AG100ATet was at 64 and 1024 µg mL^{-1}. Diarra *et al.*[22] reported that ethanol extract of *Vaccinium macrocarpon* at 0.4 and 0.8% concentration did not affect survival of *L. monocytogenes* in a cooked chicken-breast meat model. Ceruso *et al.*[23] reported that *Baphia racemosa* and *Sansevieria hyacinthoides* extracted using methanol showed the lowest value of MIC 2.5 mg mL^{-1} against *L. monocytogenes*. Susceptibility to plant extracts of these pathogenic bacteria has been documented but this is the first time that antibacterial activity of *Z. zamiifolia* stem extracts against this group of bacteria is

reported. *Z. zamiifolia* may be an alternative plant for developing new drug formulations and the effects of cytotoxicity on human cells should be studied.

CONCLUSION

The *Z. zamiifolia* stem was extracted using 5 extraction solvents. The results presented that the methanolic and ethanolic extraction were the suitable extraction solvent for *Z. zamiifolia* stem extraction. The new finding obtained from this study give novel information on antibacterial activity from *Z. zamiifolia* stem extracts against human pathogenic bacteria which benefit and useful for antibiotic drug development.

SIGNIFICANCE STATEMENT

This study discovers the novel antibacterial containing *Z. zamiifolia* stem extracts that can be beneficial for the new antibiotic drug development. This study will help the researcher to uncover the critical areas of the evaluation of the antibacterial activity of plant extracts that many researchers were not able to explore. Thus, a new application using the antibacterial activity obtained from *Z. zamiifolia* stem extracts may be arrived at.

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