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Research Article
Evaluation of the Antibacterial Activity of *Spathiphyllum wallisii* Extracts Against Human Pathogenic Bacteria

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Abstract

Background and Objective: The urgent of finding new antibiotics due to the rising of antibiotic-resistant bacteria. The plant is the main source of new antibiotic substances. The purpose of this research was to evaluate the antibacterial activity of *Spathiphyllum wallisii* extracts against nine human pathogenic bacteria. **Materials and Methods:** The stalks, leaf, rhizome and root of *S. wallisii* were extracted by using hexane, dichloromethane, ethyl acetate, ethanol and methanol. The disc diffusion assay was used to screen the antibacterial activity of *S. wallisii* extracts. Broth dilution and colorimetric assay were used to determine the Minimal inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values of extracts. **Results:** The lowest MIC values at 0.048 mg mL^{-1} were presented in the stalks extract with dichloromethane, ethyl acetate, methanol and ethanol against *B. subtilis* TISTR 008, the leaf extracted with hexane, dichloromethane, ethyl acetate, methanol and ethanol against *B. subtilis* TISTR 008; the leaf extracted with ethyl acetate, methanol and ethanol against *S. aureus* TISTR 1466, the leaf extracted with dichloromethane, ethyl acetate, methanol and ethanol against *S. aureus* PK; the rhizome extracted with methanol against *S. aureus* PK. The lowest of MBC value of 0.048 mg mL^{-1} was obtained from methanolic rhizome extract against *B. subtilis* TISTR 008. **Conclusion:** The methanolic rhizome extract of *S. wallisii* demonstrated the highest of pathogenic bacterial growth inhibition. This is the first report about the antibacterial activity of *S. wallisii* extracts that will add new information in natural drug discovery and development in industrial pharmacology.

Key words: *Spathiphyllum wallisii*, antipathogenic bacteria activity, rhizome extracts, methanolic extract, drug discovery

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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

Due to the rising worldwide of antibiotic-resistant pathogenic bacteria, therefore, the urgent need for finding new antibiotics. The year 2019 in The United States has identified more than 2.8 million antibiotic-resistant infections. The bacteria frequently develop to enhanced antimicrobial-tolerance before the antimicrobial resistance development. Bacterial resistance is the ability of a bacterium can survive in an inhibitory concentration of an antibiotic drug[1].

Natural products are a challenging source to reduce bacterial infection and resistance because containing various antibacterial compounds. The major groups of antimicrobial compounds found in plants are simple phenol, phenolic acid, quinones, tannin, coumarins, alkaloids, lectin, polypeptides[2], terpenoids, essential oils[3,4], flavones, flavonoids and flavonols[5]. Many plants had been reported on the source of natural product such *Thymus capitatus*[4], *Teucrium polium* L.[5,6], *Uapaca heudelotti*[5], *Elettaria cardamomum*[7], *Murraya koenigii*[8], *Rosmarinus officinalis* L.[9], *Suaeda maritima*[10], *Spirogyra neglecta*[11], *Spathiphyllum cannifolium*[12–16], *Spathiphyllum wallisii*[17], etc.

Spathiphyllum wallisii (Peace Lily) is the monocotyledonous ornamental plant that belongs to the family of Araceae[17]. A few previous studies reported that *Spathiphyllum cannifolium* leaf extracted with methanol and ethyl acetate have antibacterial activity against *Bacillus subtilis*[3,4]. Dhayalan *et al.*[12] reported that the chloroform *S. cannifolium* leaf extract can be inhibited *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, whereas the ethanol leaf extract inhibited only *E. coli*, *S. aureus* and *B. subtilis*, respectively. There are no previous reports about the antibacterial activity of *S. wallisii* extracts. This study aimed to evaluate antibacterial activity obtained from *S. wallisii* extracts. This is the first report that presents the information on antibacterial activity obtained from *S. wallisii* extracts against pathogenic bacteria which useful for 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) and Bacterial Agar were purchased from HiMedia (HiMedia Laboratories Pvt. Ltd, India).

Fig. 1: *Spathiphyllum wallisii*

Human pathogenic bacteria: *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* TISTR 1383 were purchased from the Thailand Institute of Scientific and Technological Research culture collection (TISTR culture collection), Thailand. *Staphylococcus aureus* PK and *Listeria* spp. were obtained from Major of Microbiology, Faculty of Science, Mahasarakham University, Thailand.

***S. wallisii* extracts preparation:** *S. wallisii* were purchased from Ornamental plant shop, Thawat Dindang, Thawat Buri, Roi Et, Thailand (Fig. 1). The stalks, leaf, rhizome and root of *S. wallisii* were separated and washed 3 times with water. Each part of *S. wallisii* sample was dried by using hot air oven (POL-EKO-APARATURA company, Wodzisław Śląski, Poland) at 50°C for 48 h. Dried plant samples were powdered using a powder grinder. Ten grams of each plant sample powder was extracted with 100 mL of different solvent (hexane, ethyl acetate, dichloromethane, ethanol and methanol). The extraction was done by shaking at room temperature for 3 h before the extracts were filtered through Whatman Filter paper No. 1. The filtrate was evaporated using a rotary vacuum evaporator (BÜCHI Labortechnik AG, Switzerland) and percent

yield was calculated[18]. Dimethyl sulfoxide (DMSO, Sigma) was added into each extract to the final concentration at 500 mg mL^{-1} before used:

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

Screening of antibacterial activity of *S. wallisii* extracts: The antibacterial activity of each extract was screened using the disc diffusion method as described by Hettiarachchi *et al.*[19]. Pathogenic bacteria were overnight cultured and adjusted the cell concentration at OD600 to 0.1 before spread onto Mueller Hinton agar (MHA). Sterile paper dish with a diameter of 0.6 mm was placed onto MHA. Ten microliters of each extract were dropped onto disc paper and DMSO was used as a negative control. The plate was allowed the extract diffusion for 15 min and plates were then incubated for 24 h at 37°C. The formation of a clearing zone around the paper disc was measured and indicated as antibacterial activity.

***In vitro* antibacterial activity of *S. wallisii* extracts:** The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were carried out by using the broth microdilution method[20]. Each *S. wallisii* extracts was twofold serial diluted with Mueller Hinton broth (MHB) in 96-well plate. The overnight pathogenic bacterial culture has adjusted the concentration at OD600 to 0.1 followed by adding 100 µL into each well of 96-well plate. Kanamycin (50 mg mL^{-1}) and DMSO were used as control. The 96-well plates were incubated overnight at 37°C. The pathogenic bacterial survival was measured using iodonitrotetrazolium chloride (INT) as an indicator[21]. Fifty microliters of 4 mg mL^{-1} INT were added in each well and the 96-well plates were incubated 37°C for 1 h. The survived pathogenic bacteria were observed from the pink color of INT whereas the well without bacterial growth remained the yellow color. The MIC value was mentioned as the lowest concentration of the plant extract that completely inhibits the bacterial growth. The MBC was considered as the lowest concentration of plant extract that kills all bacteria[22].

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

RESULTS AND DISCUSSION

***S. wallisii* extraction:** The stalks, leaf, rhizome and root of *S. wallisii* were extracted by using 100 mL of each extraction solvents. The result indicated that the highest percentage yield at 5.233% was obtained from methanolic stalk extract, followed by rhizome extracted with dichloromethane (5.154%), methanolic leave extract (5.128%) and methanolic root extract (3.892%), respectively (Table 1). This result was similar to Anokwuru *et al.*[23] that report about the percentage yield of plant extract is different depend on plant species and plant part sample.

Screening of antibacterial activity of extracts using the disc diffusion method: The diameter of inhibition zones (mm) of *Spathiphyllum wallisii* extracts against 9 pathogenic bacteria at 500 mg mL^{-1} concentration was measured using the disc diffusion method. The results presented that the largest of inhibition zones at 25 mm were presented in methanolic leaf extract against *K. pneumoniae* TISTR 1383 and root extracted with dichloromethane against *K. pneumoniae* TISTR 1383. Follow by stalk extracted with dichloromethane at 18 mm against *B. subtilis* TISTR 008 and the smallest of inhibition zone at 14 mm was obtained in methanolic rhizome extract against *B. subtilis* TISTR 008 (Table 2). This result was similar to previous stduies[12,24] that reported *S. cannifolium* (Dryand. ex Sims) Schott extracts exhibited the large zone of inhibition against *B. subtilis* and *Staphylococcus aureus*. Thus, the follow-up experiments were conducted to measure their MIC and MBC against these pathogenic bacteria.

Antibacterial activity of *S. wallisii* extracts: *In vitro* antibacterial activities of *S. wallisii* extracts were demonstrated that the lowest of Minimum Inhibitory Concentrations (MIC) values of 0.048 mg mL^{-1} were obtained from; the stalks extract with dichloromethane, ethyl acetate, methanol and ethanol against *B. subtilis* TISTR 008; the leaf extracted with hexane,

Table 1: Extraction yield of the *Spathiphyllum wallisii* extracts

	Yields (%)			
Extraction solvent	Stalks extract	Leaf extract	Rhizome extract	Root extract
Hexane	0.340	1.785	5.125	1.053
Dichloromethane	0.346	2.158	5.154	1.184
Ethyl acetate	0.486	2.631	5.006	1.295
Methanol	5.233	5.128	4.071	3.892
Ethanol	1.399	2.712	4.857	1.063

Table 2: The diameter of inhibition zones (mm) of *Spathiphyllum wallisii* extracts against nine pathogenic bacteria at 500 mg mL^{-1} concentration

		Pathogenic bacteria								
		Gram-positive						Gram-negative		
Extract	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
		Inhibition zones (mm)								
Stalks	Hexane	9	6	-	-	-	-	-	-	-
	Dichloromethane	18	17	-	-	-	-	-	-	-
	Ethyl acetate	16	15	-	-	-	-	-	-	-
	Methanol	16	15	-	-	-	-	-	-	-
	Ethanol	15	14	-	-	-	-	-	-	-
Leaf	Hexane	15	9	-	-	-	-	-	-	19
	Dichloromethane	19	18	-	-	21	-	-	-	23
	Ethyl acetate	17	18	-	-	20	-	-	-	18
	Methanol	18	9	-	-	22	-	-	-	25
	Ethanol	18	16	-	-	22	-	-	-	24
Rhizome	Hexane	-	10	-	-	-	-	-	-	-
	Dichloromethane	-	-	-	-	-	-	-	-	-
	Ethyl acetate	8	7	-	-	-	-	8	-	-
	Methanol	14	13	-	8	11	9	-	-	8
	Ethanol	-	12	-	-	-	-	7	-	-
Root	Hexane	9	6	-	-	-	-	-	-	7
	Dichloromethane	20	20	-	-	-	-	-	-	25
	Ethyl acetate	19	17	-	-	-	-	-	-	24
	Methanol	16	15	-	-	-	-	-	-	23
	Ethanol	18	16	-	-	-	-	-	-	24
	DMSO	-	-	-	-	-	-	-	-	-

- : No antibacterial activity

Table 3: MIC value of *Spathiphyllum wallisii* extracts against pathogenic bacteria (mg mL^{-1})

		Pathogenic bacteria								
		Gram-positive						Gram-negative		
Extract	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
		Minimum inhibitory concentrations (mg mL^{-1})								
Stalks	Hexane	0.097	0.780	-	-	-	-	-	-	-
	Dichloromethane	0.048	0.190	-	-	-	-	-	-	-
	Ethyl acetate	0.048	0.097	-	-	-	-	-	-	-
	Methanol	0.048	0.390	-	-	-	-	-	-	-
	Ethanol	0.048	0.097	-	-	-	-	-	-	-
Leaf	Hexane	0.048	0.190	-	-	-	-	-	-	0.78
	Dichloromethane	0.048	0.097	-	-	0.048	-	-	-	0.39
	Ethyl acetate	0.048	0.048	-	-	0.048	-	-	-	0.78
	Methanol	0.048	0.048	-	-	0.048	-	-	-	1.56
	Ethanol	0.048	0.048	-	-	0.048	-	-	-	12.50
Rhizome	Hexane	-	6.200	-	-	-	-	-	-	-
	Dichloromethane	-	-	-	-	-	-	-	-	-
	Ethyl acetate	0.190	3.120	-	-	-	-	1.56	-	-
	Methanol	6.200	6.200	-	12.5	0.048	1.56	-	-	12.50
	Ethanol	-	3.120	-	-	-	-	6.25	-	-
Root	Hexane	0.390	1.560	-	-	-	-	-	-	6.20
	Dichloromethane	0.097	6.200	-	-	-	-	-	-	25.00
	Ethyl acetate	0.390	0.097	-	-	-	-	-	-	1.56
	Methanol	0.097	0.097	-	-	-	-	-	-	3.12
	Ethanol	0.097	0.097	-	-	-	-	-	-	3.12
	Kanamycin	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.039	<0.004

Table 4: MBC value of *Spathiphyllum wallisii* extracts against pathogenic bacteria (mg mL^{-1})

		Pathogenic bacteria								
		Gram-positive						Gram-negative		
Extract	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
		Minimal bactericidal concentration (mg mL^{-1})								
Stalks	Hexane	25.00	25.00	-	-	-	-	-	-	-
	Dichloromethane	1.56	0.78	-	-	-	-	-	-	-
	Ethyl acetate	1.56	3.12	-	-	-	-	-	-	-
	Methanol	6.20	1.56	-	-	-	-	-	-	-
	Ethanol	3.12	1.56	-	-	-	-	-	-	-
Leaf	Hexane	12.50	6.20	-	-	-	-	-	-	12.50
	Dichloromethane	0.19	1.56	-	-	0.19	-	-	-	12.50
	Ethyl acetate	0.19	0.78	-	-	1.56	-	-	-	25.00
	Methanol	12.50	1.56	-	-	6.20	-	-	-	12.50
	Ethanol	0.78	0.78	-	-	6.20	-	-	-	25.00
Rhizome	Hexane	-	12.50	-	-	-	-	-	-	-
	Dichloromethane	-	-	-	-	-	-	-	-	-
	Ethyl acetate	25.00	6.20	-	-	-	-	25	-	-
	Methanol	0.048	0.39	-	3.12	3.12	6.2	-	-	3.12
	Ethanol	-	12.50	-	-	-	-	25	-	-
Root	Hexane	6.20	6.20	-	-	-	-	-	-	25.00
	Dichloromethane	12.50	6.20	-	-	-	-	-	-	25.00
	Ethyl acetate	25.00	25.00	-	-	-	-	-	-	25.00
	Methanol	6.20	6.20	-	-	-	-	-	-	25.00
	Ethanol	1.56	3.12	-	-	-	-	-	-	25.00
	Kanamycin	<0.004	<0.004	<0.004	<0.004	>25	0.019	0.019	0.039	0.625

dichloromethane, ethyl acetate, methanol and ethanol against *B. subtilis* TISTR 008; the leaf extracted with ethyl acetate, methanol and ethanol against *S. aureus* TISTR 1466; the leaf extracted with dichloromethane, ethyl acetate, methanol and ethanol against *S. aureus* PK; the rhizome extracted with methanol against *S. aureus* PK, respectively (Table 3). The most suitable *S. wallisii* part for able inhibited of bacterial growth was leaf, stalks and rhizome, respectively. The lowest of MBC value of 0.048 mg mL^{-1} was obtained from methanolic rhizome extract against *B. subtilis* TISTR 008. Follow by Minimal Bactericidal Concentration (MBC) values of 0.19 mg mL^{-1} were obtained from leaf extracted with dichloromethane and ethyl acetate against *B. subtilis* TISTR 008, extracted with dichloromethane against *S. aureus* PK, respectively (Table 4). The finding of this study was similar to Dhayalan *et al.*[12] that reported the *S. cannifolium* (Dryand. ex Sims) Schott extracts exhibited the highest inhibition against *B. subtilis*.

Results suggested that methanol is the better solvent for the extraction of antimicrobial compounds against the test organisms used in this study. The suitable *S. wallisii* part was rhizome which showed the MBC value against 6 of 9 pathogenic bacteria. The most sensitive pathogenic bacteria against extracts was presented in *S. aureus* TISTR 1466 follow by *B. subtilis* TISTR 008, *K. pneumoniae* TISTR 1383, *S. aureus* PK, *E. coli* TISTR 527, *S. epidermidis* TISTR 518 and *Listeria* spp., respectively. The most resistant pathogenic bacteria against extracts were *B. cereus* TISTR 2373 and *P. aeruginosa* TISTR 2370.

CONCLUSION

The stalks, leaf, rhizome and root parts of *S. wallisii* were extract using 5 differ extraction solvents. The results demonstrated that methanolic extraction was the suitable extraction solvent. The rhizome parts of *S. wallisii* was the most suitable part for antibacterial substances extraction. The finding of this study suggested that *S. wallisii* extracts are a potential source of antibacterial substances that benefit and useful for antibiotic drug development.

SIGNIFICANCE STATEMENT

This study discovers the novel antibacterial activity from *S. wallisii* that can be beneficial for the new 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 *S. wallisii* extracts may be arrived at.

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