ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT FROM *JASMINUM SAMBAC* AIT. FLOWER

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Abstract
The ethanolic extract from *Jasminum sambac* Ait. (Family-Oleaceae) flower was prepared to evaluate the antimicrobial activity against twelve microorganisms strains by using agar well diffusion method to determine of inhibition zone and broth microdilution method to determine the minimum inhibitory concentrations (MIC), minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC). The results revealed that the ethanolic extract from *J. sambac* showed the largest inhibition zone of 9.67±0.58 mm against *Saccharomyces cerevisiae*, the lowest MIC of 250 µg/ml against both *Staphylococcus aureus* and *Staphylococcus epidermidis* and the lowest MBC of 2000 µg/ml against both *Bacillus subtilis* and *Basillus cereus*. MIC, MBC or MFC and zone of inhibition values were compared with positive controls (ampicillin, amikacin and clotrimazole) and negative control (Dimethyl sulfoxide). Therefore, the ethanolic extract is potent to inhibit microbial growth of both Gram-negative and Gram positive bacteria and fungi.

Keywords: *Jasminum sambac*, Antibacterial activity, Antifungal activity

Introduction

Jasminum sambac Ait. (Family-Oleaceae) commonly known in Thai Medicinal Plant as Mali or Malila is a suberect or scrambling shrub (Kiritikar KR *et al.* 2003). *J. sambac* is commercially cultivated for flower and value added products like essential oils. The plant contains friedelin, lupeol, betulin, α-amyrin, ursolic acid (Rastogi RP *et al.* 1991) sambacin, jasminin, sambacoside A, sambacolignoside, quercitin, isoquercitin, rutin, kaempferol, luteolin (Upaganlawar AB *et al.* 2003), phenylmethanol, linalool, α-terpineo (Rastogi RP *et al.* 1995) and Secoirridoid glucoside- sambacoside A-G along with oleoside 11- methylester (Tanahashi T *et al.* 1988). The plant is reported to have antidiabetic (Upaganlawar AB *et al.* 2003), antitumor (Radu S *et al.* 2002), antimicrobial (Hussaini RA *et al.* 2009), antioxidant (Latif FA *et al.* 2010), anti-acne (Harisaranraj RS *et al.* 2010), suppression of puerperal lactation (Shrivastav P *et al.* 1988), A.N.S stimulating effect (Hongratanaworakit T 2010). Traditionally plant is used in fever or cough, indolent ulcer, abdominal distension, diarrhoea, lowering the blood glucose level, regulating menstrual flow, to clean kidney waste, inflamed and blood shot eyes. Root, flowers, leaves act as lactifuge, arrest the secretion of milk in the puerperal state in case of threatened abscess (Swati S *et al.* 2013). Therefore, more studies pertaining to the use extract of plants as antimicrobial agents should be highlight, exceptionally those related to the control of antibiotic resistant microbes. The objective of this research was to evaluate the potential of ethanolic extract from Thai Medicinal Plant *J. sambac* (flower) on standard microorganism strains and to compare antibiotics against drugs.

Methodology

Plant material

The flowers of *Jasminum sambac* Ait. were collected from Thai traditional drug stores. The sample was identified and compare specimen with the herbarium at Department of Applied Thai Traditional Medicine, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand.

Extraction

The air-dried and powdered plant material were extracted successively by maceration under room temperature with 95 % ethanol for 48 hours. The extract was filtrated and evaporated under reduced pressure at 50 °C by the rotary evaporator to obtain the crude extract. The extract yield was weighed, recorded and stored at -20 °C for further antimicrobial testing. The crude extract was dissolved in Dimethyl sulfoxide (DMSO) to obtain a concentration of 400 mg/ml for agar diffusion.

Microorganisms

Bacillus subtilis ATCC6633, *Staphylococcus aureus* ATCC6538P, *Escherichia coli* ATCC25922, *Candida albicans* ATCC10230 and *Saccharomyces cerevisiae* ATCC9763 were obtained from Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University; *Staphylococcus epidermidis* (Isolates), *Salmonella typhimurium* (Isolates) and *Shigella sp.* (Isolates) were from Department of Microbiology, Faculty of Science, Chulalongkorn University and *Basillus cereus* ATCC11778, *Micrococcus luteus* ATCC9341, *Salmonella typhi* (Isolates) and *Enterobacter aerogenes* ATCC13048 were from Department of Microbiology, Faculty of Sciences and Technology, Suan Sunandha Rajabhat University.

Preparation of culture media

The bacterial strains were maintained in Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB) for antibacterial testing whereas the fungal strains were maintained in Sabouraud Dextrose agar (SDA) and Sabouraud Dextrose broth (SDB) for antifungal testing.

Preparation of the inoculum

Both bacterial and fungal strains were maintained on Muller-Hinton and Sabouraud agar respectively. They were inoculated at 37 °C, for 18-24 hrs for bacteria and 24-48 hrs for fungi. Four to five of isolated colonies from the overnight culture were suspended in 0.85% of normal saline. The turbidity of the suspension was measured by using the spectrophotometer at 625 nm to obtain the absorbance of 0.08-0.10 which comparable to 0.5 Mc Farland standards. (approximately 1 x 10^8 CFU/ml) (Clonical and Laboratory Standards Institute 2009; McFarland 1907; Schwalbe R *et al.* 2007).

Determination of zone of inhibition

Antimicrobial testing was evaluated by using a slightly modified agar well diffusion method with a two-layer agar technique (Okeke MI *et al.* 2001; Mathabe MC *et al.* 2006). A 100 µl of the suspension (1 x 10^8 CFU/ml) was mixed with sterile seeded agar, then poured on the sterile base agar (Schwalbe R *et al.* 2007). The plates were allowed to dry at room temperature. Agar wells were cut from seeded agar plates by a cork borer (6 mm.) (Chantana K 2015; Anesini C *et al.*1993; Bell SC *et al.* 1968; Parekh J *et al.* 2007). Twenty microliters of plant extract of 400 mg/ml and 2 mg/ml for positive controls were transferred into each well with diameter of 6 mm. The plates were incubated at 37 °C for 18 to 24 hrs and 24 to 48 hrs for bacterial and fungal strains respectively. The antimicrobial activity was evaluated by

measuring the diameters of zone inhibition surrounding the crude extract. The zones of inhibition were measured in millimeter and the experiment was carried out in triplicates.

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The microdilution method was performed according to Rios and Recio, (Rios JL and Recio MC 2005) with slightly modification. A microbial suspension in broth was prepared by adding 10 µl of normal saline microbial suspensions to 1 ml of Muller-Hinton or Sabouraud broth. Into a sterile 96-well microplate, 50 µl of microbial suspended in broth was added to the wells containing 50 µl of plant extract (final concentrations: 7.81-4000 µg /ml with two-fold dilution), positive controls: ampicillin, amikacin and clotrimazole (final concentrations: 0.039-20 µg/ml with two-fold dilution) and negative control (DMSO). All of chemicals was prepared by diluting with broth to obtain final volume of 1 ml and incubated at 37^{o} C, for 18 to 24 hrs for bacteria and 24 to 48 hrs for fungi. The lowest concentration of plant extract inhibiting the growth of the tested microorganisms detected by the lack of visual turbidity compared to the negative control was defined as the MIC for the extract (Ramli S and Ruangrungsi N 2011). The samples of the known MIC wells were streaked onto agar plates and incubated at 37^{o} C, for 18-24 hrs for bacteria and 24-48 hrs for fungi. The least concentration with no microbial growth observed on the plate was considered as MBC or MFC value.

Results

Results obtained in the present relieved that the tested ethanolic extract from *J. sambac* flower posses potential of five gram positive bacteria and five gram negative bacteria for antibacterial activity against and two fungi for antifungal activity against. The results revealed that the ethanolic extract from *J. sambac* showed the largest inhibition zone of 9.67±0.58 mm against *Saccharomyces cerevisiae*, the lowest MIC of 250 µg/ml against both *Staphylococcus aureus* and *Staphylococcus epidermidis* and the lowest MBC of 2000 µg/ml against both *Bacillus subtilis* and *Basillus cereus*. The results were shown in Table 1-2.

Table 1 Zone of inhibition of ethanolic extract, negative and positive controls

Tested microorganisms	Zone of Inhibition (mm)				
	EJF	**Ampicillin**	**Amikacin**	**Clotrimazole**	**DMSO**
Staphylococcus aureus	7.33±0.58	46.00±1.01	11.33±0.59	20.67±0.59	NA
Staphylococcus epidermidis	8.67±0.58	26.67±0.58	15.00±0.00	24.67±0.58	NA
Micrococcus luteus	NA	45.33±0.58	14.00±0.00	33.00±0.00	NA
Bacillus subtilis	7.33±0.58	16.33±0.58	14.33±0.58	22.67±0.58	NA
Basillus cereus	7.00±0.00	18.33±0.58	15.00±0.00	22.00±0.00	NA
Escherichia coli	7.00±0.00	23.67±0.58	15.00±0.00	NA	NA
Enterobacter aerogenes	NA	10.67±0.58	10.00±0.00	NA	NA
Salmonella typhi	NA	34.67±0.58	10.00±1.00	NA	NA
Salmonella typhimurium	NA	30.67±0.58	15.33±1.15	NA	NA
Shigella sp.	7.67±0.58	29.67±0.58	16.00±1.00	NA	NA
Candida albicans	NA	NA	NA	32.00±1.00	NA
Saccharomyces cerevisiae	9.67±0.58	NA	NA	34.67±1.15	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate. EJF = Ethanolic extract of *Jasminum sambac* flower

Table 2 MIC and MBC or MFC of ethanolic extract, negative and positive controls

Sample / Tested microorganisms		MIC and MBC/ MFC				
		EJF	**Ampicillin**	**Amikacin**	**Clotrimazole**	**DMSO**
Staphylococcus aureus	MIC	250	0.039	2.5	2.5	NA
	MBC	>4000	1.25	10	>20	NA
Staphylococcus epidermidis	MIC	250	0.156	0.625	1.25	NA
	MBC	>4000	1.25	2.5	20	NA
Micrococcus luteus	MIC	NA	0.156	2.5	0.312	NA
	MBC	NA	0.312	2.5	5	NA
Bacillus subtilis	MIC	1000	>20	1.25	2.5	NA
	MBC	2000	>20	2.5	10	NA
Basillus cereus	MIC	1000	20	5	2.5	NA
	MBC	2000	20	5	20	NA
Escherichia coli	MIC	1000	2.5	2.5	NA	NA
	MBC	>4000	10	5	NA	NA
Enterobacter aerogenes	MIC	NA	>20	>20	NA	NA
	MBC	NA	5	5	NA	NA
Salmonella typhi	MIC	NA	1.25	5	NA	NA
	MBC	NA	>20	5	NA	NA
Salmonella typhimurium	MIC	NA	0.312	1.25	NA	NA
	MBC	NA	5	2.5	NA	NA
Shigella sp.	MIC	500	2.5	1.25	NA	NA
	MBC	4000	20	5	NA	NA
Candida albicans	MIC	NA	NA	NA	0.039	NA
	MFC	NA	NA	NA	0.312	NA
Saccharomyces cerevisiae	MIC	>4000	NA	NA	0.625	NA
	MFC	>4000	NA	NA	1.25	NA

NA = no activity, EJF = Ethanolic extract of *Jasminum sambac* flower

Discussion and Conclusion

The ethanolic extract from *J. sambac* flower possessed good activity against the six bacterial namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Basillus cereus*, *Escherichia coli*, *Shigella sp.* and did not exhibit activity against *Micrococcus luteus, Enterobacter aerogenes, Salmonella typhi* and *Salmonella typhimurium.* The antifungal activity the extract from this plant exhibited the antifungal activity only *Saccharomyces cerevisiae* did not show the potential on *Candida albicans*. Three positive controls of this study ampicillin and amikacin did not showed the activity against *Saccharomyces cerevisiae* and *Candida albicans* as clotrimazole not showed the inhibitory effect against *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhi*, *Salmonella typhimurium* and *Shigella sp.* (Figure 1). The ethanolic extract from *J. sambac* flower demonstrated antimicrobial activities against tested gram positive bacteria and less potent on against gram negative bacteria and fungi. The present study revealed the antimicrobial potentials among ethanolic extract from *J. sambac* flower and antibiotic drugs. The results could expand our knowledge in Thai traditional plant usages and discloses Thai traditional wisdom. Furthermore, the antimicrobial activities against pathogenic as well as antibiotic resistant microorganisms were recommended.

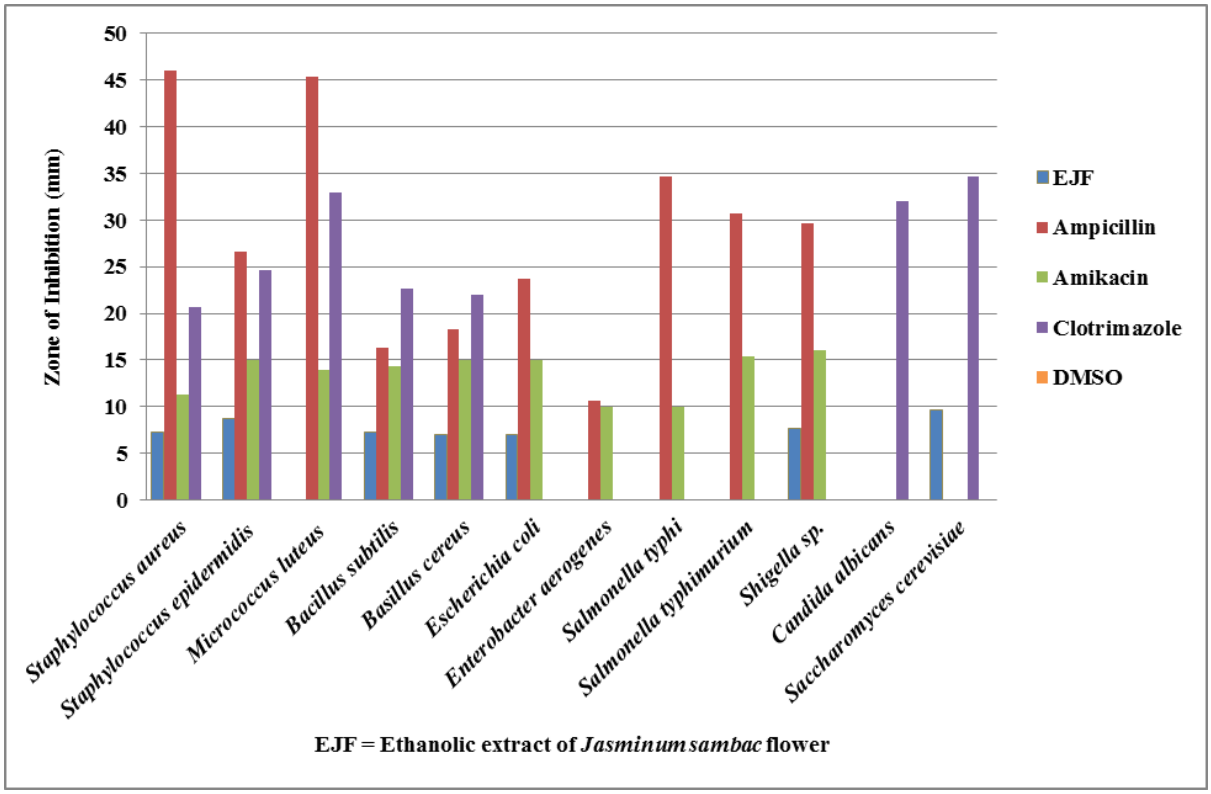

Figure 1 Spectrum of antimicrobial activity of extract, positive and negative controls

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