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Pharmacological screening of methanolic extract of *Ixora* species

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ABSTRACT

Objective: To investigate the antimicrobial activity of methanolic extracts of different parts of *Ixora* species. **Methods:** Antimicrobial activity was carried out using disc diffusion assay against fungi, gram-positive and gram-negative bacteria. **Results:** All methanolic extracts of different parts of *Ixora* species showed a broad-spectrum of antibacterial and antiyeast activities, which inhibited the growth of at least one bacterium or yeast. There was no remarkable difference between different *Ixora* species observed in this study. **Conclusions:** The significant antimicrobial activity shown by this *Ixora* species suggests its potential against infections caused by pathogens. The extract may be developed as an antimicrobial agent.

1. Introduction

Multiple drug resistance has become a very real problem in pharmacotherapeutics as there is an increasing number of diseases exhibiting various levels of drug resistance, including bacterial infections[1]. The search for new drugs to combat this difficulty is receiving much attention[2,3].

There is currently enormous surge of interest in the use, development and conservation of the medicinal plants throughout the world[4]. Malaysia is endowed naturally with a very rich plant life and the use of some of these in traditional medicines needs to be well documented[5,6]. Among the many plants of medicinal values in Malaysia, the one, which has yet to gain prominence and popularity, is the *Ixora* species. Plants used in traditional medicine have the potential to provide pharmacologically active natural products, which can be used to treat various ailments. This could be achieved by taking advantage of information available from traditional medicine and/or ethnobotanical knowledge[7].

The species belonging to the genus *Ixora* are amongst the plants used in Indian traditional Ayurvedic system of medicine for a variety of ailments. Leaves are used to treat diarrhoea; roots in hiccough, fever, sore, chronic ulcers, and skin diseases; while flowers in catarrhal bronchitis and dysentery[8]. The genus *Ixora* ia a genus of the family Rubiaceae. Eventhough they are widely distributed in Malaysia, only little is known about its chemistry and biological activity.

Therefore, the objective of this preliminary study was to evaluate and compare the antimicrobial activity of methanolic crude extract of various parts of different *Ixora* species.

2. Materials and methods

2.1 Microorganisms

Staphylococcus aureus (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus mirabilis* (*P. mirabilis*), *Escherichia coli* (*E. coli*), *Acinetobacter calcoaceticus* (*A. calcoaceticus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter anitratus* (*A. anitratus*), *Bacillus licheniformis* (*B. licheniformis*), *Staphylococcus epidermidis* (*S. epidermidis*), *Citrobacter preundii* (*C. preundii*), *Salmonella typhi* (*S. typhi*), *Burkholderia pseudomallei* (*B. pseudomallei*), *Erwinia* sp., *Bacillus cereus* (*B. cereus*), *Bacillus subtilis* (*B. subtilis*), *Candida albicans* (*C. albicans*), *Rhodotorula rubra* (*R. rubra*), *Cryptococcus neoformans* (*C. neoformans*), *Trichoderma viride* (*T. viride*), *Rhizopus* sp., *Mucor* sp.,

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Penicillium sp., *Fusarium solani* (*F. solani*), *Fusarium oxysporium* (*F. oxysporium*), *Trichophyton rubrum* (*T. rubrum*), *Microsporum canis* (*M. canis*), *Trichophyton mentagrophytes* (*T. mentagrophytes*), *Aspergillus niger* (*A. niger*) and *Aspergillus flavus* (*A. flavus*) were the test organisms used and obtained from the Fermentation and Enzyme Technology Laboratory, University Science of Malaysia. The bacterial isolates were maintained on nutrient agar slants at 37 ℃ while fungi including the yeasts were maintained on Sabouraud dextrose agar slants at 30 ℃.

2.2. Plant materials

Ixora plants (leaves and flowers) were obtained from Universiti Sains Malaysia Campus, Penang, Malaysia and authenticated by a taxonomist at the School of Biological Sciences, Universiti Sains Malaysia. The herbarium samples were deposited at the Herbarium of the School of Biological Sciences, USM.

2.3. Preparations of the crude extract

The dried plant parts were exhaustively extracted with 200 mL of methanol in a Soxhlet apparatus for 4 h. The extract was then concentrated in a rotary evaporator under reduced pressure.

2.4. Antimicrobial activity

The antimicrobial activities of the different extract preparations from *Ixora* species were determined following the method described by Lachumy *et al*[9] with slight modifications.

Test microorganisms were cultured on a respective growth media and removed aseptically with an inoculating loop and transfered to a test tube containing 5.0 mL of sterile distilled water. Sufficient inoculums were added until the turbidity equal to 0.5 McFarland standards. 1 mL of the suspension was added to the 15 mL of medium agar before setting aside the seeded agar plates to solidify for 15 min. To screen the antimicrobial activity, Whatman's filter paper No. 1 discs of 6 mm diameter were used. Each sterile disk, containing 100 mg of the extract per mL from the *Ixora* species, was placed on the surface of the seeded plates. The plates were incubated at 37 ℃ overnight and examined for zones of growth inhibition.

3. Results

Antimicrobial activity results of methanolic extracts of the

Table 1
Antimicrobial activities of crude methanolic extract of *Ixora* species on various microorganisms.

Microorganisms		RF	RFL	DRF	DRL	SWF	SWL	PF	P FL	LYF	LYL	C	M
Bacteria	*S. aureus*	12	30	8	14	20	14	16	12	18	12	28	ND
	P. aeruginosa	12	25	14	20	14	14	14	16	20	16	25	ND
	P. mirabilis	16	14	16	–	16	–	14	8	16	–	24	ND
	E. coli	12	14	12	10	12	10	12	10	12	10	31	ND
	A. calcoaceticus	18	24	18	16	18	16	18	16	25	–	29	ND
	K. pneumoniae	16	12	16	–	14	–	14	–	–	–	24	ND
	A. anitratus	12	10	12	10	12	14	12	10	10	10	28	ND
	B. licheniformis	10	18	10	12	8	8	10	12	10	8	22	ND
	Micrococcus sp.	–	20	–	22	20	14	–	14	20	–	27	ND
	S. epidermidis	12	14	12	12	10	12	14	12	10	–	23	ND
	C. preundii	15	20	15	28	18	16	14	16	22	10	28	ND
	B. subtilis	8	20	8	14	8	8	12	14	12	–	30	ND
	Erwinia sp.	–	14	–	16	–	16	–	14	14	–	26	ND
	B. cereus	14	14	16	12	14	16	14	15	13	12	21	ND
Yeasts	*C. albicans strain* 1	12	12	10	12	12	10	10	12	10	12	ND	24
	C. albicans strain 2	12	12	12	14	10	10	10	10	10	12	ND	30
	C. albicans strain 3	10	12	10	12	14	10	10	12	10	14	ND	26
	R. rubra	18	20	14	20	20	20	20	18	20	12	ND	22
	C. neoformans	12	12	12	14	12	14	14	14	10	12	ND	21
Fungi	*T. viride*	–	–	–	–	–	–	–	–	–	–	ND	22
	Rhizophus sp.	–	–	–	–	–	–	–	–	–	–	ND	21
	Mmucor sp.	–	–	–	–	–	–	–	–	–	–	ND	22
	Penicillium sp.	–	–	–	–			–	–	–	–	ND	21
	Fusarium sp.	–	–	–	–			–	–	–	–	ND	22
	T. rubrum	–	–	–	–	–	–	–	–	–	–	ND	21
	M. canis	–	–	–	–	–	–	–	–	–	–	ND	22
	T. mentagrophytes	–	–	–	–	–	–	–	–	–	–	ND	21
	F. oxysporium	–	–	–	–	–	–	–	–	–	–	ND	22
	A. niger	–	–	–	–	–	–	–	–	–	–	ND	21
	A. flavus	–	–	–	–	–	–		–	–	–	–	23

The values (average of triplicate) are diameter of zone of inhibition at 100 mg/L/disc. C: chloramphenicol; M: miconazole; RF: red flower, RFL: red flower species leaf; DRF: dark red flower; DRL: dark red flower species leaf; SWF: sandal wood color flower; SWL: sandal wood color flower species leaf; PF: pink flower; PFL: pink flower species leaf; LYF: light yellow flower; LYFL: light yellow flower species leaf.

different parts of the *Ixora* species were given in Table 1. Ten methanolic extracts tested showed antibacterial activity against at least one bacterium or yeast. Almost all the methanolic extracts exhibited antimicrobial activity against gram-negative and gram-positive bacterial strains. The different methanolic extract of flower and leaves of *Ixora* species showed a broad-spectrum antimicrobial activity. Apart from bacteriostatic, anti-yeast effects, none of the methanolic extracts exhibited anti-fungal activity. Only the light yellow color flower leaf exhibited less anti-microbial activity.

4. Discussion

The main objective of this study was to evaluate and compare the ability of different *Ixora* species to produce anti-microbial activities. There are no remarkable difference between different *Ixora* species in this study. In our study, *Ixora* species had a wide variety of antimicrobial activity against pathogenic microorganisms.

Our antimicrobial activity result was comparable with study done by Annapurana *et al* and Latha *et al*[10,11] but they only used *Ixora coccinea* species against bacteria and yeast cell. Annapurana *et al*[10] tested the ether and methanol extracts of *Ixora cocciniea* leaves for antimicrobial activity. They reported that all the extract tested showed antimicrobial activity against the entire test organisms including *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*.

The detected anti-microbial activities are in line with the uses of the *Ixora* species in traditional medicine. As traditional healers use water as a solvent for preparation of plant extracts, methanolic extract procedure may be relevant, at least in terms of validation of the use of plants in traditional medicine[7].

Plants used in traditional medicine are assumed to be safe due to the long-term use by traditional healers[7]. Information about the safety and effective use of medicinal plants is difficult to find due to the lack of rigorous clinical studies and limited toxicological data available[12].

There are many reports in the literature regarding the biological activity of *Ixora* species[13-23]. The present work has shown that *Ixora* species was a potentially good source of antimicrobial agent and that further investigation is worthwhile to isolate and evaluate biologically active compounds from the crude extract. Further purification of the active compounds and *in vivo* evaluation of their antimicrobial activity, along with toxicity studies of the potential extracts from *Ixora* species, are therefore suggested as further studies.

Conflict of interest statement

We declare we have no conflict of interest.

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