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Screening of potential antimicrobial activity from *Hymenocallis littoralis* (Jacq.) Salisb

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Abstract

The screening of antimicrobial activity namely disk diffusion and minimum inhibitory concentration (MIC) were carried out using *Hymenocallis littoralis* (Jacq.) Salisb plant's extracts with twelve (12) different parts of juvenile and flowering stages against twelve (12) microbes. Gram positive bacteria (*Micrococcus spp, Bacillus subtilis, Bacillus thuringiensis, Staphylococcus aureus*), Gram negative bacteria *Escherichia coli* (normal), *Escherichia coli* (hospital strain) *Pseudomonas aeruginosa, Salmonella spp, Proteus mirabilis, Klebsiella pneumoniae*), and fungal strains (*Candida albicans, Aspergillus niger*). Flowering stage explant produced pronounced inhibition of microbe's growth compared to the juvenile stage. Roots from the flowering stage extracts produced highest activity against *Bacillus subtilis* (zone of inhibition was 29.0 $\pm$ 0.04mm), followed by the old leaves from flowering stage extracts against *Micrococcus spp* and *Bacillus thuringiensis*. In addition, stems extracts from the juvenile stage produced highest zone of inhibition at 11.0 $\pm$ 0.24mm against *Asperagillus niger*. Based on the best disc diffusion results, further investigation was carried out by performing the minimal inhibitory concentration (MIC) analysis on extracts of young leaves, old leaves, stems, bulbs, roots, flower and flower stalks against *Micrococcus spp*, *Escherichia coli* hospital strain, *Candida albicans* and *Asperagillus niger.* Best MIC was obtained at 6.25mg/ml from the juvenile roots and flowering old leaves against *Micrococcus spp;* flowering old leaves and stems against *Escherichia coli* hospital strain and juvenile old leaves, flowering old leaves and stems against *Candida albicans*.

Keywords: Hymenocallis littoralis (Jacq.) Salisb plants, antimicrobial, Disc diffusion, MIC.

Introduction

Opportunistic diseases in the globalization of the world contributed huge number of increasing the survival rate of patients. The prime agent that leads to these diseases is pathogenic microorganisms[19]. Several diseases have been treated by the administration of plant extracts from medicinal plants.[9 and 15] Therefore, many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants[9]. For example, extracts of *Garcinia kola* seeds can potentially be useful in the treatment of staphylococcal wound infections [18].

The traditional uses of medicinal plants in health-care practices are providing clues to new areas of research and hence its importance is now well recognized [11]. *Hymenocallis littoralis* (Jacq.) Salisb commonly known as 'Spider Lily' is a bulbous, herbaceous plant from the family of Amaryllidaceae[14]. The plant is distributed by the sea and in swamps in tropical, sub-tropical, and temperate regions throughout the world[10]. Throughout the history of *Hymenocallis littoralis*, several alkaloids had been discovered from its bulb. The first alkaloid lycorine was proven to have antineoplastic, cytotoxic and antiviral properties [1, 12 and 16].

The present investigation was made to evaluate the antimicrobial activity of *Hymenocallis littoralis* (Jacq.) Salisb wild plant explants. The objectives of present study are to determine the disc diffusion and minimum inhibition concentration (MIC) of *Hymenocallis littoralis* wild (Jacq.) Salisb plant extracts.

Materials and Methods

Plant Source

Hymenocallis littoralis (Jacq.) Salisb plants used in this study (Figure 1) were obtained from Penang Botanical Gardens, Malaysia. The plants were cut into their part namely young leaf, old leaf, stem, bulb and root for juvenile stage; and young leaf, old leaf, stem, bulb, root, flower and flower stalk for flowering stage. Plant materials were washed with distilled water and dried in an oven at 35-40°C for 7 days.

Plant Extract Preparation

Two (2) stages of of *Hymenocallis littoralis* (Jacq.) Salisb of juvenile and flowering were collected, dissected, and washed with clean sterile water. Then, the explants were surface sterilized with 10% Sodium Hypochlorite solution and rinsed with sterile distilled water before dried in an oven at 45°C for 48 hours. Finally, explants was ground using mortar and pestle into fine powder sample.

Chloroform and Methanol Extractions

The extraction procedure used in this study was modified from the procedure of alkaloid extraction[6, 7 and 13]. One (1) gram of dried samples was ground using pestle and mortar, before adding 20 ml of solvent 3:1 ($CHCl_3$: MeOH). The extracts were then sonicated by using analog ultrasonic cleaner (WUC-A02H WiseClean Ultrasonic Cleaner, DAIHAN Sciencetific Co., Ltd. Sungbuk-Gu, Korea) for 15 minutes. The homogenate was filtered through four layers of miracloth and centrifuged at 12 000 X g for 10 min at 4 °C.

Test Solution and Disk Preparation

Test solution was prepared with known weight of crude extracts, and dissolved in 5% dimethyl sulphoxide (DMSO). Whatman No. 1 sterile filter paper discs (6 mm) were impregnated with 20µl of this extract (corresponding to 100 mg/ml of crude plant extract) and allowed to dry at room temperature.

Microorganisms

Ten (10) bacteria strains used were Gram positive bacteria, namely *Micrococcus spp, Bacillus subtilis, Bacillus thuringiensis* and *Staphylococcus aureus;* and

Gram negative bacteria namely *Escherichia coli* (hospital strain), *Pseudomonas aeruginosa, Pseudomonas aeruginosa, Pseudomonas aeruginosa, Salmonella spp, Proteus mirabilis* and *Klebsiella pneumoniae.* Two (2) fungal pathogens used were *Candida albicans* and *Aspergillus niger.* These standard strains were obtained from the Microbiology Laboratory stock, School of Biological Sciences, Universiti Sains Malaysia. All bacteria were maintained on Nutrient Agar (NA) at 37°C and fungi on Potato Dextrose Agar (PDA) at 28 °C.

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined using the broth dilution method 4. The crude Hymenocallis littoralis (Jacq.) Salisb extract was dissolved in methanol to obtain the initial concentration as 100 mg/ml. Two-fold dilution was performed from the initial test concentration and each tube was inoculated with 500 ìl suspension containing 108 CFU/ml of bacteria and 104 spore/ml of fungi. The test tube for bacteria was incubated at 37 °C for 24 hours and at 28 °C for 48 hours for fungi. MIC was determined as the lowest concentration of the extract inhibiting the visual growth of the test cultures in the tubes based on turbidity.

Results and Discussion

Twelve (12) samples crude extracts of different explants from juvenile and flowering stages from *Hymenocallis littoralis* (Jacq.) Salisb wild plant were used to screen the antimicrobial activity namely the juvenile stage; young leaf, old leaf, stem, bulb and root and flowering stage; young leaf, old leaf, stem, bulb, root, flower and flower stalk (Table 1). Meanwhile, twelve (12) microbes (Table 1) were used to test all the samples, namely Gram positive bacteria (*Micrococcus* spp, *Bacillus subtilis*, *Bacillus thuringiensis*, *Staphylococcus aureus*), Gram negative bacteria (Escherichia coli [normal]), *Escherichia coli* (hospital strain) *Pseudomonas aeruginosa*, *Salmonella* spp, *Proteus mirabilis*, *Klebsiella pneumoniae*, and fungal strains (*Candida albicans* and *Asperagillus niger*).

Overall, flowering stage explants showed pronounced inhibition of microbe's growth of the cultures compared to the juvenile stage with different range on inhibiton zone (Table 1). Most probably, the highest amount of secondary metabolite was presented such as an alkaloid in mature plant was the main reason for these results obtained. These results were supported in the statement by Borek 4. that the mature fruits were the richest organs for daidzein concentration in Porzana cinerea. In other hand, Godjevac (2004) 8 and Slavica (2004)19 were reported the present of flavonoids from flower of their selected research plants.

Stems extracts from the juvenile stage against *Aspergillus niger* produced highest zone of inhibition at 11.0 + 0.24mm. In addition, roots from the flowering stage extracts produced highest activity against *Bacillus subtilis* (zone of inhibition was 29.0 + 0.04mm), followed by the old leaves from flowering stage extracts against *Micrococcus* spp and *Bacillus thuringiensis*. The highest zone of inhibition from this investigation was contrast different with the disc diffusion results from20. the extraction of the flower of *Musa acuminata* whereas the highest zone of inhibition from their study was 22mm against *Staphylococcus aureus* and the lowest zone inhibiton was 12mm against *Escherichia coli*. Meanwhile, similar result of the highest disc diffusion was reported that leaves extracts of *Euphorbia hirta* against *Micrococcus* sp. and the lowest zone of inhibition was 9 mm from the flower plant extract against *Proteous mirabilis*[2]. The zone of inhibition for negative control (methanol) was zero. However, larger clear zones were observed on the plates with 30µg/ml of kanamycin tested on *Micrococcus* spp and 30µg/ml penicillin (antibiotics) tested on *Bacillus* compared to the methanol crude extract (Table 1).

Minimal inhibitory concentrations (MIC) was carried out on selected extracts from young leaves, old leaves, stems, bulbs, roots, flower and flower

stalks against selected microbes, namely the *Micrococcus* spp, *Escherichia coli* hospital strain, *Candida albicans* and *Aspergillus niger*. Best MIC was obtained at 6.25mg/ml from the juvenile roots and flowering old leaves against *Micrococcus* spp; flowering old leaves and stems against *Escherichia coli* hospital strain; and juvenile old leaves, flowering old leaves and stems against *Candida albicans* (Table 2). This was significantly different with the MIC results obtained by other researchers 2, 20. Meanwhile, the best MIC value was at 1.56 mg/ml tested on *Musa acuminata* extract against *Staphylococcus aureus* 20. In addition, the best MIC value was reported about 3.13 mg/ml extracts of Euphorbia hirta against *Escherichia coli* and *Candida albicans* 2.

The significant antimicrobial activity from disc diffusion and MIC shown by these extracts suggested that *Hymenocallis littoralis* (Jacq.) Salisb could be a potential remedy against infections caused by *Candida albicans* and *Escherichia coli* hospital strains.

Conclusion

Overall, flowering stage explants showed pronounced inhibition of microbe's growth compared to the juvenile stage. Roots from the flowering stage extracts produced highest activity against *Bacillus subtilis*, followed by the old leaves from flowering stage extracts against *Micrococcus* spp and *Bacillus thuringiensis*. In addition, stems extracts from the juvenile stage against *Aspergillus niger* produced highest zone of inhibition. Best MIC was obtained at 6.25mg/ml from the juvenile roots and flowering old leaves against *Micrococcus* spp; flowering old leaves and stems against *Escherichia coli* hospital strain; and juvenile old leaves, flowering old leaves and stems against *Candida albicans*.

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Microorganisms (Antibiotic)	Zone of Inhibition of disc diffusion (mm)											
	Extracts											
	1a	2a	3a	4a	5a	1b	2b	3b	4b	5b	6b	7b
Micrococcus spp (Penicillin)	25 (38.3)	24 (35)	24.3 (23)	24.3 (41.3)	25 (24)	27 (20)	28 (45.3)	24 (45)	26 (39)	24 (32)	25 (32)	26.3 (33)
Escherichia coli(normal) (Kanamycin)	21 (22)	24 (24)	24.3 (22)	24 (33)	20 (24)	23 (20)	23.3 (35.7)	23 (19)	21 (19)	19 (21)	23 (20)	24 (20)
Escherichia coli(hospital) (Kanamycin)	21.3 (22)	20 (23)	20 (24)	22 (23)	21 (23)	21.3 (20)	24 (19)	24 (17)	22 (20)	23 (20)	24 (18)	20 (15)
Bacillus subtilis (Penicillin)	19 (22)	20 (22)	19 (23)	21.3 (23)	24 (23.3)	20 (18)	21 (17)	20 (19)	26 (19)	19 (18)	20 (14)	22 (15)
Pseudomonas aeruginosa (Kanamycin)	13 (22)	20 (22)	23 (25)	22 (25)	17 (25)	15 (23)	21 (16)	21 (20.7)	21 (25)	18 (22)	21 (21)	21 (21)
Salmonella spp (Kanamycin)	21 (22)	23 (22)	20 (25)	28 (24)	23.3 (24.3)	25 (20)	23 (18)	24 (20)	24 (20)	26 (20)	22 (22)	23 (21)
Bacillus thuringiensis (Penicillin)	23 (33.3)	23 (35)	21 (23)	27 (35)	27 (35)	26 (20)	23 (28)	22 (30)	27 (33)	29 (28)	27 (22)	23 (25)
Proteus mirabilis (Kanamycin)	20 (23)	23.3 (24.5)	25 (26)	23 (25)	24 (23)	22.3 (19)	23 (25)	24 (20)	24.3 (18)	22 (19)	24 (21)	25 (25)
Staphylococcus aureus (Vancomycin)	20 (23)	21 (25)	21 (25)	22 (23.3)	23 (27)	22 (15)	23 (21)	20 (21)	19 (24)	20 (21)	23 (17)	19 (24)
Klebsiella pneumonia (Kanamycin)	24 (23.5)	20 (23)	23 (24)	22 (23.3)	24.3 (23.3)	20 (18.7)	24.3 (18)	23 (20)	22.3 (20)	24 (19)	26.3 (24)	24 (24)
Candida albicans (Mecanazole nitrate)	24 (23)	24 (20)	23 (24)	27 (23)	26 (27)	22 (15)	23 (22.7)	24 (20)	27 (18.3)	25 (22)	22 (18)	23 (19)
Aspergillus niger (Amphotericin)	15 (23)	13 (23.5)	11 (23)	17 (23.5)	17 (26.3)	12 (21)	15 (22)	19 (20)	20 (19.3)	19 (20)	23 (22)	23 (20)

Table 1: Antimicrobial activity by using disk diffusion technique. The values (average of triplicate) are diameter of zone of inhibition at 100mg/ml crude extract of *Hymenocallis littoralis* (Jacq.) Salisb, 30 ìg/ml each of antibiotic tested.

Microorganisms	MIC (mg/ml) Extract											
	1a	2a	3a	4a	5a	1b	2b	3b	4b	5b	6b	7b
Micrococcus spp	12.5	-	-	-	6.25	-	6.25	-	-	-	-	-
Escherichia coli(normal)	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli(hospital)	-	-	-	-	-	-	6.25	6.25	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella spp	-	-	-	12.5	-	-	-	-	-	-	-	-
Bacillus thuringiensis	-	-	-	12.5	12.5	-	-	-	-	-	-	-
Proteus mirabilis	-	-	100	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumonia	-	-	-	-	-	-	-	-	-	-	-	-
Candida albicans	12.5	6.25	12.5	12.5	25	12.5	6.25	6.25	12.5	25	12.5	12.5
Aspergillus niger	-	-	-	-	-	50	12.5	12.5	50	25	50	12.5

Table 2: Antimicrobial activity of minimum inhibitory concentration (MIC) by using broth dilution method. The values are in mg/ml of crude extract of Hymenocallis littoralis (Jacq.) Salisb.

Figure 1: *Hymenocallis littoralis* (Jacq.) Salisb plant