Poster:

Studies on antibiotic compounds of methanol extract of *Curculigo latifolia* dryand

[1]Lim Sheh Hong* and [1]Darah Ibrahim

[1]Industrial Biotechnology Research Laboratory (IBRL), School of Biological Sciences Universiti Sains Malaysia, 11800 Penang Malaysia. *Corresponding author email: limshehhong77@gmail.com

Abstract. A research was conducted to study the effect of different parts of the extracts from *Curculigo latifolia* Dryand plant. They were from the roots, stems and leaves by using methanol as the extraction solvent. The antimicrobial activity of methanol extract from *Curculigo latifolia* Dryand was then performed on various tested bacteria, yeasts and fungi. The results revealed that the extracts possessed antimicrobial activities on all of tested bacteria and yeasts. However, all the tested fungi exhibited resistant against all the different parts of *Curculigo latifolia* Dryand extracts. The minimum inhibitory concentration (MIC) and minimum lethality concentration (MLC) of the *Curculigo latifolia* Dryand extracts against bacteria and yeast cells were determined and the mode of action of the roots extract on the cells was studied by means of microscopy.
Key words: *Curculigo latifolia*, minimum inhibitory concentration, minimum lethality concentration, methanol extract

Introduction

There is an urgent need for the development of new antibiotic substances in the light of recent reports of microbial resistance to some of the well known antibiotics (Spanggaard *et al*., 1993; Dempsey, 1993). One avenue of search is to screen microbial secondary metabolites for their antibiotic activity. Higher plant produce diverse chemical compound with different biological activities (Hamburger & Hostettmann, 1991). It is believed that those compounds have played an important ecological role. They can work as chemical defenses against insect, herbivores and also microorganisms (Harbone, 1990, Palakawong *et al*., 2010). Therefore, this has encouraged the researchers to search the higher plants for various biological activities such as antimicrobial activities.

Curculigo latifolia Dryand is a large tufted herbaceous plant from Amarylliaceae family. This plant has been found abundantly distributed in both Peninsular Malaysia and Borneo. In Borneo, traditionally the leaves are pounded and wrapped around the knees to ease joint pains. Also, previous studies have shown that this plant possess some medicinal value. Hence, the present work was carried out to investigate the antimicrobial properties from *Curcurigo latifolia* Dryand.

Materials and Methods

Preparation of the extract

Curculigo latifolia plant was collected from several locations including Teluk Kumbar, Sungai Ara and Bayan Lepas, Penang, Malaysia. The leaf, root and stem of *C. latifolia* Dryand were washed, dried and ground to powder form. Then, 100 g of ground sample was extracted using Soxhlet apparatus with 500 ml methanol as solvent, at 30 °C for 48 hours. The resultant extract was then concentrated to dryness in a rotary evaporator at 40 °C. The stock solution containing 1000 mg/ml (w/v) was prepared in the methanol and then further diluted in distilled water.

Microorganisms

The microorganisms used in this study consisted of bacteria, yeast and fungi. The bacterial strains were grown and maintained on nutrient agar (NA) slants, while yeast and fungi on Sabouraud dextrose agar (SDA) slants. The inoculated agar slants were incubated at 37°C for bacteria, and 30°C for fungi and yeast.

Determination of minimal inhibitory concentration (MIC) and minimum lethality concentration (MLC)

MIC was determined by the liquid dilution method. Serial dilution of extract was prepared to obtain final concentrations between 1.0 mg/ml to 100.0 mg/ml. Extract at different concentration were added aseptically into test tube containing 1500 µl sterile Sabouraud dextrose broth (SDB) for yeast and nutrient broth (NB) for bacteria. Inoculums containing 4 x 10^6 cell/ml were inoculated into respective test tubes. The inoculated test tubes were incubated

at 37°C for 24 hours for bacteria and 30°C, 48 hours for yeast. The lowest concentration which did not show any growth of the tested microorganism after macroscopic evaluation was determined as the MIC. However, the MLC was recorded as the lowest concentration of the extract that gave complete inhibition of colony formation of the test microorganisms on agar plate.

The morphological study of the C. albicans cells after treated with the root extract

One ml of 4 x 10^6 cell/ml suspension was added into Sabouraud glucose broth containing root extract with a final concentration of 25.0 mg/ml (MLC). The inoculated broth was incubated at at 30°C for 48 hours. A small volume of the inoculated broth was withdrawn at various time intervals and was fixed for scanning electron microscopy viewing.

Results and discussion

The methanolic extract of different part of *C. latifolia* plant showed antibacterial and antiyeast activities against the entire tested bacteria and yeast. It was observed that no antifungal activities against all the tested fungal. The resistance of fungal species against the extracts could be due to the thicker cell walls and contain higher percentage of chitin (Madigan & Martinko, 2006). The results obtained suggested that the extracts were effective against prokariotic compared to eukariotic cells. The liquid dilution method for susceptibility test of the methanolic extract of *C. latifolia* is shown in Table 1.It was noted that higher concentration of extract was needed to kill the microorganism then inhibited the growth of microorganisms. Lim *et al*., (2006), have reported MLC value was higher compared to MIC value in their studied.

Anticandidal activity of *C. latifolia* root extract on yeast cells has not been widely studied, but it can be postulated that its anticandidal activity is by disrupting the structure of the cell membrane and inhibiting the normal budding process due to the destruction of the membrane integrity. These conditions can be observed further in Figure 1. The *C. albicans* cells showed a smooth surface and budding stage when not exposure to the extract. However, after exposure to root extract the cells exhibited notable alteration in the cell membrane with formation of invagination, cavitated cells and eventually collapsed the cells.

Table 1 : Antimicrobial activities of methanolic extract of *C. latifolia* Dryand

Test microorganism	Zone of inhibition (mm)			Root extract	
	root	Stem	Leaf	MIC (mg/ml)	MLC (mg/ml)
Bacteria					
Bacillus cereus	17	15	9	12.5	25
Bacillus subtilis	15	16	16	12.5	25
Enterobacter aerogenes	15	14.5	13	25	50
Erwinia sp.	15	16	13	25	50
Klebsiella sp.	17	16	14	25	50
Pseudomonas sp.	22	19.5	17	12.5	25
Staphylococcus aureus	18	18	18	6.25	12.5
Yeast					
Candida albicans	25	22	21	12.5	25
Cryptococcus neoformans	12	14	10	25	50
Fungi					
Aspergillus flavus	-	-	-	ND	ND
Aspergillus niger	-	-	-	ND	ND
Microsporum canis	-	-	-	ND	ND
Trichyton mentagrophytes	-	-	-	ND	ND

ND: not determined

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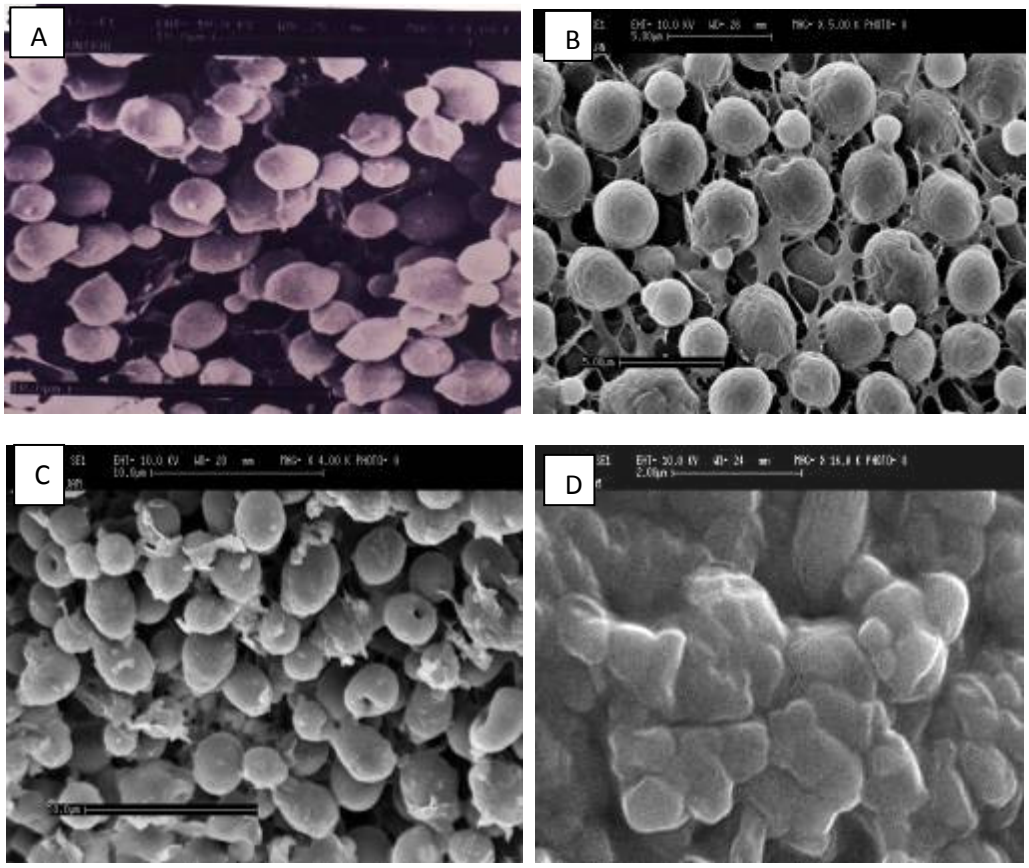

Figure 1. SEM photomicrograph of *Candida albicans* cells after exposure to roots extract of *Curculigo latifolia* Dryand. (A), Control; (B), 12 hours; (C), 24 hours; (D), 36 hours.

Conclusions

The present investigation showed that there are antibacterial and antiyeast properties in methanolic extract of *C. latifolia* Dryand. However, it needs to be further processed to obtain pure compound(s) that can then be tested for antimicrobial activities.

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