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Short Communication

Antimicrobial alkaloids from the leaves of *Pandanus amaryllifolius*

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ABSTRACT

Chemical investigation on the crude base of the *Pandanus amaryllifolius* leaves led to the isolation and identification of pandamarilactone-1 (**1**), pandamarilactone-32 (**2**), pandamarilactonine-A (**3**), and pandamarilactonine-B (**4**). Their structures were elucidated based on 1H and 13C NMR and in comparison with the literature data. Compound **3** was found to be the most active among the four isolates with an MIC of 15.6 µg/mL and MBC of 31.25 µg/mL against *Pseudomonas aeruginosa*. This is the first report on the antimicrobial activity of the isolated alkaloids from the genus *Pandanus*.

INTRODUCTION

The family Pandanaceae or the screw pine family is composed of four monocotyledon genus: the *Pandanus*, *Freycinetia*, *Sararanga*, and *Martellindendron* (Callmander, 2001; Callmander *et al.*, 2003). The largest among the four, the genus *Pandanus* of about 700 species, is prevalent in tropical and sub-tropical areas, especially on the Pacific islands, Malaysian islands and Australia. Several of the *Pandanus* species are recognized as medicinal plants and used in traditional medicines. The *P. amaryllifolius* commonly known as the fragrant screw pine because of its scented leaf is used to refresh the body, reduce fever, and relieve indigestion. The oil of the leaves is used as purgative, as a treatment for leprosy, stimulant and cures headaches and rheumatism (Cheeptham, 2002; Quisumbing, 1978). The root decoction showed hypoglycemic activity and 4-hydroxybenzoic acid was identified as the active compound (Peungvicha *et al.*, 1998). Phytochemical analysis on the leaves and roots of *P. amaryllifolius* had elaborated mostly the presence of alkaloids (Tan *et al.*, 2010a; Tan *et al.*, 2010b). However, there is a dearth on the pharmacological activity on the identified alkaloids. Based on the ethnopharmacological activities, the isolation and identification of alkaloids and their antimicrobial activities, including the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), will be reported in this paper.

MATERIALS AND METHODS

The Plant Material

Fresh leaves of *P. amaryllifolius* was collected in March 2014 and identified by Miss Ophelia Laurente, botanist of the UST Herbarium. A voucher specimen with accession number USTH-3728 was deposited at the UST Herbarium.

Extraction and isolation

The air-dried and ground leaves (1.1 kg) were percolated with distilled MeOH for a total of 7 L for 3 consecutive days. The combined filtrate was concentrated under reduced pressure to obtain the crude extract (195 g). The crude extract was dissolved in 1M H_2SO_4 and partitioned with DCM thrice.

The acid layer was basified to pH 8 with the addition of Na_2CO_3. The basified solution was extracted with DCM and the collected organic layers were concentrated under reduced pressure to obtain the crude alkaloidal extract. The crude alkaloidal extract was initially subjected to column chromatography using increasing increments of MeOH in $CHCl_3$. TLC of the collected fractions resulted to five pooled fractions, PA-1 to PA-5.

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PA-1 was subjected to column chromatography using increasing increments of EtOAc and hexane to obtain three pooled fractions (PA-1A to PA-1C) after TLC. Purification of PA-1B using hexane/EtOAc (4:6) yielded **1** (20 mg) and **2** (10 mg). PA-2 was subjected to column chromatography (thrice) using hexane/ EtOAc (4:6) resulted in purification of **3** (11 mg) and **4** (10 mg).

Antimicrobial Assay

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the different extracts were determined using microwell assay. Extracts were diluted with DMSO to a concentration of 1 mg/ml, placed in microwells, then serially diluted (1:2) into 8 wells to a final volume of 100 µL for each test organism. Three bacteria were used for the assay: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853. A 100 µL of bacterial suspension (1.5×10^8 CFU/mL) was added to each well and incubated at 37^oC for 24 hours. The concentration in the last well with no growth after 24 hours was reported as the minimum inhibitory concentration (MIC).

All wells with no growth were then subcultured into nutrient agar (NA) plates to determine the minimum bactericidal concentration. The lowest concentration of extract which did not show bacterial growth in the NA plates after 24 hours was reported as the minimum bactericidal concentration (MBC). All setups were done in triplicate for each extract.

RESULTS AND DISCUSSION

Chromatographic purification of the crude base of *P. amaryllifolius* leaves led to the identification of pandamarilactone-1 (**1**) (Nonato *et al*., 1993), pandmarilactone-32 (**2**) (Nonato *et al*., 1993), pandamarilactonine-A (**3**) (Takayama 2002), and pandamarilactonine-B (**4**) (Takayama 2002) (Fig. 1). Their structures were identified based on NMR analyses and in comparison with the literature data. The compounds **1**–**4** have also been previously identified from *Pandanus dubius* (Tan *et al*., 2010c).

The presence of piperidine- (Nonato *et al*., 1993), pyrrolidinone- (Sjaifullah and Garson, 1996), pyrrolidine- (Takayama *et al*., 2002), and indolizidine-type (Cheng *et al*., 2015) alkaloids have been previously identified from the genus *Pandanus*. However, no biological activity has been associated yet with those alkaloids. To address this fact and utilizing the ethnopharmacological activities associated with *P. amaryllifolius*, the crude base and the isolates have been subjected to antimicrobial assay using the microtiter plate diffusion method. Three organisms namely *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used to determine the minimum inhibitory concentration (MIC) (Table 1) and minimum bactericidal concentration (MBC) (Table 2).

Table 1: Minimum Inhibitory Concentration (MIC) of the different isolates.

	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
1	500	500	250
2	125	500	250
3	**62.5**	**15.6**	**250**
4	500	500	250
Crude base	500	62.6	250

Table 2: Minimum Bactericidal Concentration (MBC) of the different isolates.

	Escherichia coli	*Pseudomonas aeruginosa*	*Staphyllocoocus aureus*
1	>500	>500	500
2	250	>500	500
3	**125**	**31.25**	**500**
4	>500	>500	500
Crude base	>500	125	500

Results indicated that pandamarilactonine-A (**3**) was found to be the most active among the four isolates. The crude base was also found to exhibit an activity against *P. aeruginosa*.

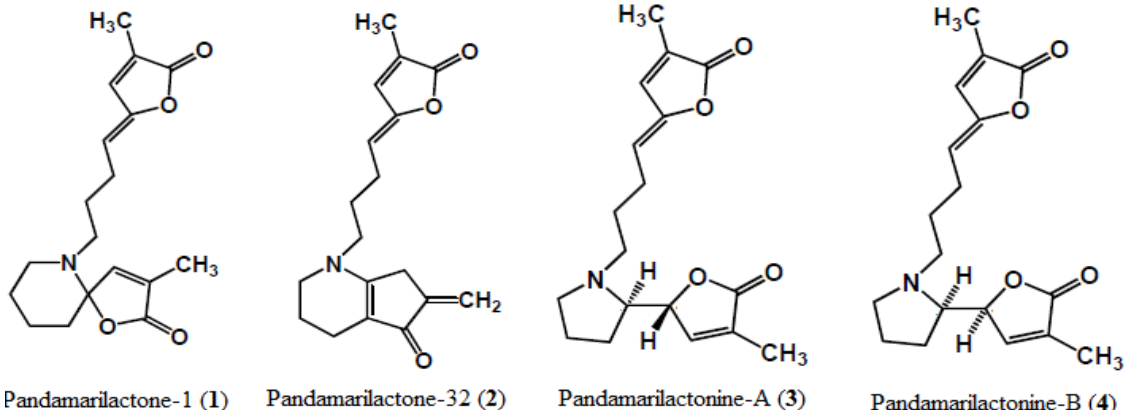

Fig. 1: Structure o compounds 1-4.

CONCLUSION

Re-investigation on the leaves of *P. amaryllifolius* had isolated the piperidine (**1** and **2**) and pyrrolidine (**3** and **4**) type alkaloids. This is the first report that their MIC and MBC antimicrobial activities have also been determined against three organisms *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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