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Letter to the Editor

Ganoderma boninense isolated from Sabah, Malaysia exhibits potent antibacterial activity against clinically important bacterial pathogens

Sir,

Ganoderma boninense is a white rot fungus, which mostly can be found in oil palm estates in Southeast Asia. It is an economically devastating pathogen causing major losses on oil palm's profit.

To date, numerous research have been done on *G. boninense*, but they are mainly focused on developing effective control tools in the form of chemicals or biological control agents (Alexander et al., 2017a), study on oil palm defence mechanism (Azura et al., 2016), the fungal pathogenesis (Alexander et al., 2017b), early detection (Alexander et al., 2014), and the molecular studies (Chong et al., 2011). Meanwhile, there are increasing number on other research field of *G. boninense* which include the study on the fungal metabolites (Alexander et al., 2014) and on pharmaceutical properties (Ismail et al., 2014; Ma et al., 2014). However, despite of all the works done on *G. boninense*, to date, there is no single report on the fungal antibacterial activity against clinically important bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica*, and *Streptococcus pyogenes* that are increasingly contribute to nosocomial-caused infections. In the present work, we have isolated a fungal fruiting body (Figure 1A) compromising an oil palm tree in local oil palm plantation in Sabah, Malaysia. The fruiting body was cultured in our laboratory and molecularly identified according to the protocols described by Chong et al. (2011). Molecular analysis revealed that the

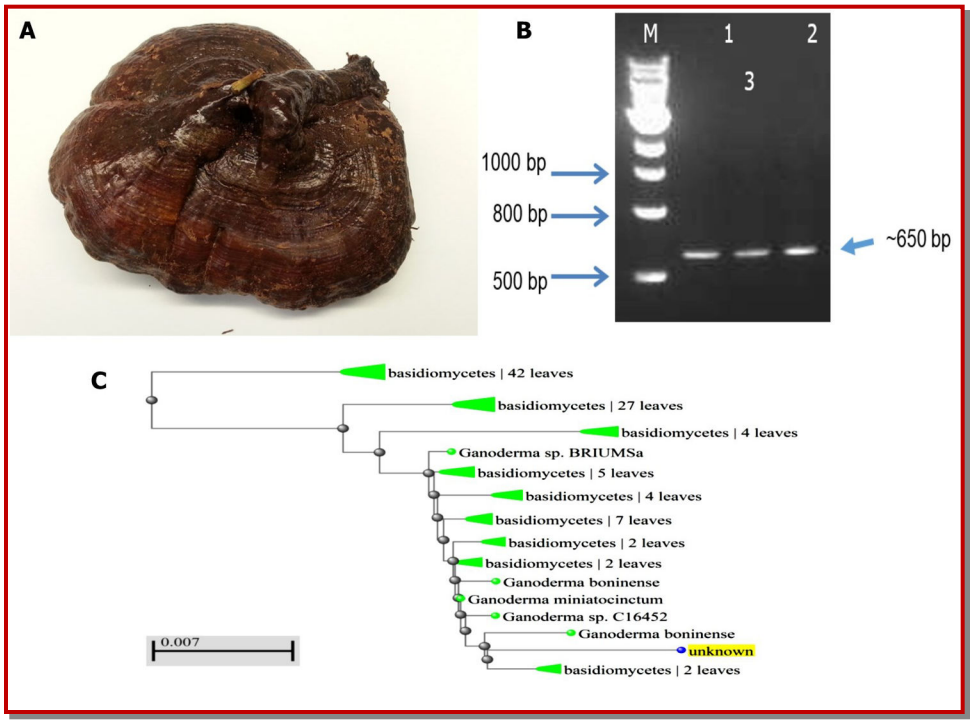

Figure 1: A) The fungal fruiting body obtained from oil palm tree; B) Polymerase Chain Reaction (PCR) analysis of the fungal fruiting body's DNA based on method described by Chong et al. (2011) shows that the PCR product is around 650 bp; C) Phylogenetic Tree analysis (using Basic Local Alignment Search Tool, BLAST, NCBI) of the fungal PCR product DNA sequence (highlighted in yellow) shows that the fungi identified as *G. boninense* species

Table I

Antibacterial activity of *G. boninense* extracts against clinical isolates of bacterial pathogens

Strain†	Zone of inhibition (mm)					
	Aqueous extract#	Methanol extract#	Acetone extract#	Chloroform extract#	Hexane extract#	Tetracycline
1	Not detected	7.7 ± 0.6^{cd}	7.7 ± 0.6^{cd}	9.3 ± 0.6^{fg}	Not detected	17.3 ± 1.5
2	Not detected	Not detected	Not detected	7.0 ± 0.0^{ab}	Not detected	15.7 ± 0.6
3	Not detected	Not detected	Not detected	7.0 ± 0.0^{ab}	Not detected	15.3 ± 0.6
4	Not detected	8.0 ± 0.0^{de}	7.7 ± 0.6^{cd}	11.3 ± 0.6^{i}	Not detected	23.7 ± 1.5
5	Not detected	7.7 ± 0.6^{cd}	Not detected	7.3 ± 0.6^{bc}	Not detected	17.3 ± 1.5
6	Not detected	7.7 ± 0.6^{cd}	7.7 ± 0.6^{cd}	9.7 ± 0.6^{gh}	Not detected	21.7 ± 1.5
7*	Not detected	7.0 ± 0.0^{ab}	Not detected	7.7 ± 0.6^{cd}	Not detected	15.7 ± 1.6^{k}
8*	Not detected	Not detected	Not detected	Not detected	Not detected	11.3 ± 1.6^{fgi}
9*	Not detected	Not detected	Not detected	Not detected	Not detected	12.0 ± 1.6^{gij}
10*	Not detected	7.3 ± 0.6^{bc}	7.7 ± 0.6^{cd}	9.3 ± 0.6^{h}	Not detected	16.0 ± 1.6
11*	Not detected	7.3 ± 0.6^{bc}	Not detected	7.3 ± 0.6^{cd}	Not detected	14.7 ± 1.6^{k}
12*	Not detected	7.3 ± 0.6^{bc}	7.0 ± 0.0^{ab}	8.7 ± 0.6^{fg}	Not detected	13.3 ± 0.6^{jk}

†Standard bacterial strains = 1) *E. coli*, ATCC 35218; 2) *K. pneumoniae*, ATCC 1705; 3) *P. aeruginosa*, ATCC 9027; 4) *S. aureus*, ATCC 25923; 5) *S. Enterica*, ATCC 14028, 6) *S. pyogenes* ATCC 19615, and *clinical bacterial isolates = 7) *E. coli*; 8) *K. pneumoniae*; 9) *P. aeruginosa*; 10) *S. aureus*; 11) *S. Enterica*; 12) *S. pyogenes*. #Extracts are in crude form; concentrations and the extracts amount loaded into the disc were standardized to 2 mg/mL and 100 µg respectively. Pure culture of the *G. boninense* fruiting body was obtained according to Chong et al. (2011). The amount loaded into each disc is 30 µg with concentration 1 mg/mL. ND= Antibacterial activity was not detected. None of the clinical bacterial isolates is Tetracycline resistant. The inhibition data are statistically different at $p<0.05$ unless stated with same letter

fungal fruiting body is belong to *G. boninense* species as shown in Figure 1B and Figure 1C. Remarkable anti-bacterial activity from the identified *G. boninense* extracts was observed against the standard and clinical bacterial isolates as summarized in Table I. Chloroform extract of *G. boninense* gives the broadest spectrum of antibacterial activity against both standard and clinical isolates of bacterial pathogens. For standard bacterial isolates, the greatest inhibition was observed on *S. aureus*, ATCC 25923 (11.3 ± 0.6 mm), followed by the inhibition on *S. pyogenes*, ATCC 19615 (9.7 ± 0.6 mm) and *E. coli*, ATCC 35218 (9.3 ± 0.6). The weakest activity was observed against *K. pneumoniae*, ATCC 1705 and *P. aeruginosa*, ATCC 9027 (7.0 ± 0.0 mm size of inhibition for both isolates). Meanwhile, for the clinical bacterial isolates, the greatest inhibition was observed on *S. aureus* (9.3 ± 0.6 mm size of inhibition) followed by *S. pyogenes* (8.7 ± 0.6 mm size of inhibition) and *E. coli* (7.7 ± 0.6 mm size of inhibition). No antibacterial activity was observed on water and hexane extracts of *G. boninense* against both the standard and clinical bacterial isolates, while two clinical bacterial isolates, *K. pneumonia* and *P. aeruginosa* were not susceptible to any of *G. boninense* extracts but to tetracycline. From this work, we found that the chloroform and methanol extracts of *G. boninense* by some par give broader spectrum of antibacterial activity against the tested bacterial pathogens compared to other extracts. This work also suggest that *G. boninense* might bearing potent antibacterial agent against important nosocomial infections-related bacterial pathogens, but right solvents system and extraction procedures are crucial to extract them out. One of *Ganoderma* species, *G. lucidum* is well known to exhibits potent medicinal potential including antibacterial activity (Iftekhar et al., 2011). This report confirmed that *G. boninense*, as wood decaying fungi like *G. lucidum* can also exhibit potent antibacterial potential. In-depth investigation is necessary to identify the responsible antibacterial compounds and the exact antibacterial mode of action against the selected bacterial pathogens.

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