Author’s response to reviews

Title: Anti-Methicillin-resistance Staphylococcus aureus (MRSA) compounds from Bauhinia kockiana Korth. and their mechanism of antibacterial activity

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Author’s response to reviews:

Reviewer reports:

Muhammad Rashid Khan, PhD (Reviewer 1): The authors have evaluated the extract or isolated chemicals B. kockiana against the MRSA strains. the results indicated the higher activity of the extract as compared to the pure compounds. Last paragraph of the discussion require more refinement. Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

Response: Replace p. 10, line 15 to 36, last paragraph

Compound 1 and 2 were less potent than extract and positive control. The potency of compound 1 and 2 decreased when they are tested individually as pure compounds. This could be explained
that synergistic interaction between compound 1 and 2 with other phytochemicals present in extract/fractions. Numerous studies have reported that plant polyphenol could synergistically interact with each other to exhibit stronger antibacterial activities [25, 35, 36]. Potency of phytochemicals could be improved when they are combined in treatment, since multiple sites in bacteria are targeted. Phytochemicals could alter the outer membrane permeability, inhibit multidrug resistance efflux pumps, function as active sites modifiers and β-lactamase inhibitors [36, 37]. Basri et al found that antibacterial activity of oxacillin against MRSA is stabilised with gallic acid, as gallic acid will function as additive [35]. Combination of oxacillin with alkyl gallates (methyl to decyl gallates) could result in stronger inhibitory effect [25].

Surajit Debnath (Reviewer 2): The work analyses a new plant for its bioactivity and it is a good effort. When published works are less for a plant it is also a challenging task. The authors may address these issue for better impact of the work:

(a) The authors have described well about the plant. However, since the plant is relatively new the authors must add specific details of the already published reports. The sentences "This plant is used as ethnomedicine for treating various diseases. In Sarawak, Eastern Malaysia, the roots of B. kockiana is used by Kelabit ethnic group for treating gonorrhoea, nervous debility, insomnia as well as fatigue [16, 17]. The bark and root infusions are used to treat toothache [14" and the sentences "it has been reported that this plant exhibited anticancer activity [13], possesses strong antioxidant properties and consists of very high total phenolic content [14, 15]": are somewhat rudimentary in terms of scientific literature. The authors may describe how the earlier studies focuses on the plants bio activity.

Response: p. 5, from line 54, added extra literature review [14]. Researches on the bioactivities and phytochemicals of B. kockiana are relatively new and not many literatures are available. Our previous studies have reported that B. kockiana could exhibit anticancer activity. It is reported that B. kockiana could exhibit anticancer activity towards various types of cell lines, including
MCF-7, PC-3 cells, LNCaP, HCT-116 and DU145 cells [13]. Antioxidant activities of B. kockiana has also been studied. Our studies reported that B. kockiana flowers and leaves consisted very high total phenolic content, 4440 to 7540 mg gallic acid equivalent/100 g, and exhibited strong free radical scavenging and ferric reducing antioxidant power [14, 15].

(b) The Fractionation and isolation of bioactive compounds section if supported by a schematic diagram it will be easy to understand.

Response: Schematic diagram of fractionation and isolation included (Fig 2).

Photographs of elution of various fractions may be provided since these are newly studied.

Response: Photographs of elution of fractions are not available

Data generated by Spectral methods may be given in the graphical form as peaks.

Response: All spectrum are attached as supplementary files.

(c) a particular interest is to visualize the inhibition zones in the diffusion assay. Best pictures of the inhibition zone produced by various fraction will make the paper more appealing.

Response: Figure 3 included as attachment

(d) A simple step by using various concentration of fractions increasingly in the zone assay will indicate the dose dependent behavior of the studied fractions when accompanied by a simple regression analysis. This will add on to the statistics described in the paper.

Response: Explanation from authors
Disc diffusion assay is a qualitative assay where the inhibition activities of the extracts and fractions are evaluated. However, only antibacterial compounds which can only diffuse through the agar can be evaluated. The diameter of the inhibition zone does not reflect the potency of the activities. Hence statistical analysis is not suitable.

Sections to be updated in manuscript

• Addition/Change in Figure number in main text

p. 6, line 40 methanol to give rise to 2 (52 mg) (Fig 2). The purities of 1 and 2 were ……

p.7, line 20 was measured (Fig 3) and the results were expressed as mean of three independent…. 

p. 9, line 44 x MICs of ethyl acetate extract and compounds (Fig 4). Bacterial cell membrane was severely ….

• Update in p. 10 Declarations (after List of abbreviations)

Ethics approval and consent to participate

Not applicable.

Funding

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Acknowledgements
The authors are thankful to Monash University Malaysia for financial support and Ms LuYii Ying for contributing the flower samples.

List of figures (p.15)

Figure 1 Bauhinia kockiana Korth with its bright orange-red magnificent inflorescences

Figure 2 Schematic diagram of fractionation and isolation of compound 1 and 2

Figure 3 Inhibition zone of fractions exhibit antibacterial activity in disc diffusion assay

Figure 4 Scanning electron microscopy (SEM) photograph of MRSA treated with ethyl acetate and isolated active compounds of B. kockiana flower at 2 × MIC (A) Control (B) treated with ethyl acetate extract (C) treated with compound 1 (D) treated with compound 2