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Letter to Editor

Comparative study of antibacterial activity between *Cynodon dactylon* crude and solid phase extraction extracts against selected bacterial pathogens

Sir,

Cynodon dactylon is a type of perennial grass that possesses great medicinal values. Due to numerous reports on usage of *C. dactylon* as traditional anti-infection agent, this grass has been intensively studied for its antibacterial properties. Previous studies shown *C. dactylon* killed some common bacterial pathogens including common nosocomial-caused infection pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Abdullah et al., 2012; Abdullah et al., 2013).

Although the antibacterial activity reported is considerably strong, however, the activity is much depending on the extraction protocols. Our present study compared the antibacterial activity of *C. dactylon* crude and Solid Phase Extraction (SPE) flush fraction extracts against *Bacillus cereus, Bacillus subtilis, E. coli, Klebsiella spp., P. aeruginosa, S. aureus, Streptococcus pyogenes* and *Streptococcus pneumonia* using disc diffusion and broth micro-dilution bioassays.

Disc diffusion bioassays of the *C. dactylon* crude and SPE extracts showed different extent of antibacterial activity against the selected bacterial pathogens (**Table I**). In general, *C. dactylon* SPE extracts showed stronger antibacterial activity against the bacterial pathogens compared to plant crude extracts. It is observed that the size of inhibition for the crude extracts is between 7.0 ± 0.0 mm to 10.0 ± 1.0 mm for ethanol extract and 7.0 ± 0.0 to 12.0 ± 1.0 mm for ethyl acetate extract. Meanwhile, size of inhibition for the flush fraction of SPE extract is ranging from 8.0 ± 0.0 mm to 15.7 ± 0.6 mm for ethyl acetate and 8.0 ± 0.0 mm to 13.0 ± 0.0 mm for ethanol SPE extracts. The minimum inhibitory concentration (MIC) expressed as the lowest extract concentration at which no visible growth was observed and measured via turbidity in broth. The MIC values for the crude extracts are ranging from 50-60 mg/mL for ethanol crude extract while 40-60 mg/mL for ethyl acetate crude extract. The MIC study revealed remarkable antibacterial properties from the SPE extracts. The SPE flush fraction from both ethanol and ethyl acetate showed lower MIC values compared to the crude extracts with the values for ethyl acetate SPE extract range from 15-30 mg/mL while 10-20 mg/mL for ethanol SPE extract, two fold from crude extracts MIC values implies that the SPE extracts possess stronger antibacterial activity. The phytochemical constituents of

Table I: Comparison of antibacterial activity (measured by diameter of inhibition in mm and Minimum Inhibitory Concentration, MIC values) between *C. dactylon* ethanol and ethyl acetate crude and Solid Phase Extraction (SPE) extracts

Tested microbial pathogens	*C. dactylon*							
	Ethanol crude extract		Ethanol SPE extract (flush fraction)		Ethyl acetate crude extract		Ethyl acetate SPE extract (flush fraction)	
	Inhibition* (mm)	MIC# (mg/mL)	Inhibition* (mm)	MIC# (mg/mL)	Inhibition* (mm)	MIC# (mg/mL)	Inhibition* (mm)	MIC# (mg/mL)
Bacillus cereus	9.0 ± 0.0	50	13.0 ± 0.0	10	12.0 ± 1.0	40	15.7 ± 0.6	15
Bacillus subtilis	7.0 ± 0.0	50	8.0 ± 0.0[a]	10	8.0 ± 0.0[a]	50	11.0 ± 0.0	20
Escherichia coli	8.3 ± 0.6[a]	60	8.0 ± 0.0[a]	20	8.0 ± 0.0[a]	60	9.0 ± 0.0	30
Klebsiella spp.	8.3 ± 0.6[a]	60	7.0 ± 0.0	20	8.0 ± 0.0[a]	60	8.0 ± 0.0[a]	30
Pseudomonas aeruginosa	8.0 ± 0.0	60	7.0 ± 0.0	20	9.3 ± 0.6[a]	50	10.0 ± 0.0[a]	30
Staphylococcus aureus	9.0 ± 1.0[a]	50	9.0 ± 0.0[a]	10	10.0 ± 0.0[a]	40	10.0 ± 0.0[a]	15
Streptococcus pyogenes	10.0 ± 1.0	60	11.0 ± 1.0	15	8.0 ± 0.0[a]	60	8.0 ± 0.0[a]	25
Streptococcus pneumonia	7.3 ± 0.6[a]	60	8.0 ± 0.0[a]	20	7.0 ± 0.0[a]	60	10.0 ± 0.0	20

*Values presented are means of three replicates ± SD; each disc loaded with approximately 60 µL or 6 mg per disc of plant extract; n.d = antibacterial activity not detected during the bioassay; CHL= Chloramphenicol (1 mg/mL, 30 µg per disc). All values of inhibition of different extracts on respective bacterial pathogens are significantly different at p=0.05 based on Tukey-test except superscripted with same letter[a]; #MIC was recorded as the concentration (mg/mL) that resulted in total inhibition of all replicates after 24 hours at 37°C

C dactylon had been comprehensively discussed in some previous work (Abdullah et al., 2012; Abdullah et al., 2014). The use of SPE in this study is aiming to further purify the active compounds of *C. dactylon* from ethanol and ethyl acetate crude extracts by the mean of removing most of the impurities through the selective SPE column sorbent. In this SPE system, the sorbent matrix is designed to retain polar compounds based on three mechanisms; 1) π-π bonding, 2) hydrogen bonding and dipole-dipole interactions and 3) hyrophobic interaction (Żwir-Ferenc and Biziuk, 2006). Previously, SPE was used for plant extracts for further purifying and concentrating the bioactive components by selectively separating the plant compounds from the crude mixture through the SPE sorbent; which ultimately enhance the antimicrobial effect (Escuredo et al., 2012). Through SPE, the bioactive compounds from *C. dactylon* are further concentrated and purified which gives better antibacterial effect.

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