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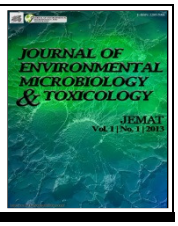

Short communication

Antibacterial Activities of *Agave angustifolia* and *Pittosporum ferrugineum*

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HISTORY
Received: 30 September 2013
Recieved in revised form: 27 November 2013
Accepted: 26 December 2013
Available online: 3 January 2014

KEYWORD
Agave angustifolia
Pittosporum ferrugineum
Antibacteria
Extraction

Abstract

Methanolic extracts and liquid – liquid partition extracts of *Agave angustifolia* and *Pittosporum ferrugineum* were tested against *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Streptococcus pneumoniae (S. pneumoniae), Enterobacter aerogenes (E. aerogenes), Bacillus cereus (B. cereus), Pseudomonas aeruginosa (P. aeruginosa)* and *Salmonella typhi (S. typhi)* for their antibacterial activity. Ethyl acetate extract (EAE) of Agave angustifolia exhibited strongest activities against S. aureus, S. pneumoniae, B. cereus, P. aeruginosa and S. typhi, while aqueous extract (AQE) of Pittosporum ferrugineum exhibited strongest antibacterial activities against S. aureus, S. pneumoniae, B. cereus, and P. aeruginosa.

INTRODUCTION

The history of natural product as antibacterial agents can be traced back during the end of World War II, where microbial derived secondary metabolites such as penicillin and streptomycin were being the notable examples known to the West [1]. Plants generally have bioactive compounds that serve mainly as self defense [2.3]. These bioactive compounds, also known as secondary metabolites or phytocomponents, were reported to possess therapeutic effects and were being manipulated by many researchers in producing useful drugs. Tropical rainforest is a vast reservoir of potential drug species as it provides valuable compounds and the potential for developing newer compounds is enormous [4].

Phytocomponents such as cardiac glycosides [5], terpenes [6], saponin [7] and flavonoids [5,8,9] were found in numerous species of Agave which exhibited biological activities as antioxidants [9], anti–inflammatory [10], and anticancer [11]. Various phytocomponents including carotenoids, sesquiterpenes glycosides, farnesyl glycosides, triterpenoid saponins and their derivatives were also hugely distributed among species in the genus Pittosporum, which contributed in activities such as cytotoxic and molluscidal [12]. In this study the antibacterial activity of *Agave angustifolia* and *Pittosporum ferrugineum* extracts against *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus),* Streptococcus pneumoniae (S. pneumoniae), Enterobacter aerogenes (E. aerogenes), Bacillus cereus (B. cereus), Pseudomonas aeruginosa (P. aeruginosa) and Salmonella typhi (S. typhi) were reported.

MATERIALS AND METHODS

Plant Materials

Agave angustifolia was collected in the compounds of Universiti Malayasia Sabah, Kota Kinabalu; while *Pittosporum ferrugineum* was collected from Ranau, Sabah. Plant samples were dried with the temperature not exceeding 50˚C and were then grinded. Powdered samples were stored in air – tight containers in dry places. Specimen for herbarium was also prepared and selected plant samples were deposited at Institute for Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah.

Extracts preparation

For crude methanolic extracts (ME), powdered samples were soaked with absolute methanol (99.8% v/v, Fisher) with the ratio of 1:10 (w/v) (sample: solvent). Extracts were then filtered, concentrated and dried in vacuo. Standard solvent extraction methods for liquid – liquid extraction (LLE) were performed as outlined by Harbone, [13]. Six LLE partition extracts generated were ethyl acetate extract (EAE), hexane extract (HE), chloroform extract (CE), chloroform – methanol extract (CME), butanol extract (BE) and aqueous extract (AQE). Extracts were dried in vacuo. All dried extracts were reconstituted back into methanol with the concentration of 100mg/ml.

Table 1. Antibacterial activities of *Agave angustifolia* and *Pittosporum ferrugineum* extracts.

Samples (mg/ml) \ Bacteria		*S. aureus*	*S. pneumoniae*	*B. ereus*	*E.coli*	*S. Typhi*	*P. Aeruginosa*	*E. Aerogenes*
		Crude Methanolic Extract						
Agave angustifolia	100	8.75±0	8.5±0	7.5±0	0	0	11.5±0	6±0
	50	8.5±0	8.5±0	6.75±0	0	0	11.5±0	0
	40	8.5±0	8.5±0	6.5±0	0	0	11.25±0	0
	30	8.5±0	8.5±0	6±0	0	0	11±0	0
	20	8.5±0	8±0	0	0	0	10.5±0	0
	10	8±0	8±0	0	0	0	9±0	0
		Liquid-Liquid Partition Extract Of Crude Methanolic						
	CE	9.5±0	8.5±0	7.5±0	0	9±0	8±0	0
	CME	0	0	7±0	0	9±0	8.5±0	0
	HE	10.5±0	9.5±0	8±0	0	8±0	13±0	0
	EAE	13.5±0	8±0	9±0	0	7.5±0	13.75±0	0
	AQE	0	0	0	0	7.75±0	8±0	0
	BE	0	7.5±0	0	0	0	8±0	0
		Crude Methanolic Extract						
Pittosporum ferrugineum	100	10.5± 0	9.5± 0	7.5± 0	-	-	11.75± 0	-
	50	10.5± 0	9.5± 0	7.5± 0	-	-	11.5± 0	-
	40	9.5± 0	8± 0	6.5± 0	-	-	10.75± 0	-
	30	9.5± 0	7.5± 0	6.5± 0	-	-	10.5± 0	-
	20	-	-	-	-	-	9± 0	-
	10	-	-	-	-	-	9± 0	-
		Liquid-Liquid Partition Extract Of Crude Methanolic						
	CE	8± 0	8± 0	8.5± 0	-	-	9± 0	8± 0
	CME	7± 0	7± 0	7± 0	-	-	9± 0	7.5± 0
	HE	8± 0	7.5± 0	-	-	-	7.5± 0	8± 0
	EAE	6.5± 0	7± 0	6.5± 0	-	-	-	7.5± 0
	AQE	9.5± 0	9± 0	-	-	-	-	8± 0
	BE	7.5± 0	7.5± 0	-	-	-	-	8± 0
Control		-	-	-	-	-	-	-
Ampicillin		28.2±1.4	32.6±6.4	11.5±0.5	13.2±0.8	16.7±0.7	3.7±0.7	8.4±0.6

Notes: CE – chloroform extract; CME – chloroform-methanol extract; HE – hexane extract; EAE – ethyl acetate extract; AQE – aqueous extract; BE – butanol extract

Test Organisms

Three gram – positive bacteria (*S. aureus, S. pneumoniae and B. cereus*) and four gram – negative bacteria (*E. coli, E. aerogenes, P. aeruginosa and S. typhi*) were used in this study.

Disc Diffusion Assay

Screening for antibacterial was performed using disc diffusion assay. Whatmann papers No 3 discs (6mm in diameter) impregnated with extracts were placed on agar, which was inoculated with the specific bacteria used in every screening test. Approximately 20µl of each samples were pipetted on the disc with final concentration of 10-100 mg/ml. Ampicillin (0.25ug/ul) was used as the positive control while the extraction solvents were used as negative control. Plates were then incubated overnight at 37˚C. Observations were carried out based on the diameter of the inhibition zones (mm) on the media. All tests were performed in triplicates; the mean and standard deviation of the inhibition zones recorded were calculated.

RESULTS AND DISCUSSION

Crude methanolic extracts of Agave angustifolia and Pittosporum ferrugineum were able to inhibit the growth of *S. aureus, S. pneumoniae, B. cereus,* and *P. aeruginosa. E. aerogenes* was only inhibited by *A. angustifolia* but only at the concentration of 30-100mg/ml. Both extracts however, failed to inhibit the growth of *E. coli* and *S. typhi* (Table 1). Similar results were observed with the screening of LLE extracts. For *A. angustifolia*, the most potential LLE extract was ethyl acetate extract (EAE) which exhibited the highest inhibition zones especially against *S. aureus* (13.5±0mm) and *P. aeruginosa* (13.75±0mm). But for *P. ferruguineum,* the most potential LLE extract was chloroform extract which exhibited larger inhibition zones (8± 0 to 9± 0mm) and more bacteria can be inhibited if compared with other LLE extracts.

CONCLUSION

Both *Agave angustifolia* and *Pittosporum ferrugineum* have phytocomponents which possess antibacterial activities. In A. angustifolia, the components are likely to be non – polar, while in P. ferrugineum, the components are likely to be moderately polar.

ACKNOWLEDGEMENT

This study was supported by Ministry of Science, Technology and Inovation, Malaysia (MOSTI) under Science Fund grant (02-01-10-SF0107).

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