Antibacterial activity of leaf extract of torch ginger (*Etlingera elatior*) against *Pectobacterium carotovorum* and *Xanthomonas campestris* pv. *campestris*

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Introduction

Torch ginger (*Etlingera elatior* (Jack) R.M. Smith) is a plant in the Zingiberaceae family. It is a native specie and wide cultivation in South East Asia. Although, the literature on *E. elatior* was mainly focused on their antioxidant properties of the extracts, few studies have discovered antimicrobial properties of this specie. Previous studies showed that methanolic flower extract of *E. elator* encouraged antibacterial activities against *Staphylococcus aureus*, *Bacillus thuringiensis*, *B. subtilis*, *Escherichia coli*, *Salmonella* sp., *Micrococcus* sp., and *Proteus mirabilis* (Lachumy et al., 2010). In addition, methanolic leaf extract of *E. elatior* also inhibited *B. cereus*, *M. luteus,* and *S. aureus* (Chan et al., 2007). However, it has not been revealed that the antibacterial activity of torch ginger against plant bacterial pathogens. Therefore, the purpose of the present study is to obtain the antibacterial activity of leaf of torch ginger extracts which are sequentially extracted by several solvents against plant pathogenic bacteria; *Pectobacterium carotovorum* and *Xanthomonas camprestris* pv. *campestris.*

Methods

1. Test bacteria

P. carotovorum and *X. campestris* pv. *campestris* were cultured in NB and shacking incubated at 250 rpm at 37°C for 24 h.

2. Plant extraction

Torch ginger leaf was washed with water and cut into small pieces then dried at 50°C in a hot air oven for 24 h. Five hundred grams of the dried leaf were sequentially maceration extracted by 4,500 mL of hexane, dichloromethane, acetone, ethanol, and methanol. Each extraction procedure was carried out at room temperature for 3 days and then was filtered through Whatman no. 1. The filtrate was evaporated using Rotary Evaporator. The solvent free extract of 50 mg/mL was then prepared by re-suspending it in the original solvent.

3. Antibacterial assay

Antibacterial activity was determined by the agar well diffusion. Pesticide, Clear (0.25 g/L) was a positive control.

4. Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC)

MIC and MBC were determined by the broth dilution method with modification. MIC was carried out using test tubes filled with 1 mL of MHB medium and 1 mL of extract, each with set concentration of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09 and 0.00 mg/mL. The 10^6 CFU/mL of each bacterial indicator was added into the test tube, and then incubated at 37°C for 24 h. The lowest concentration that did not show any visible growth was considered as MIC. The broth in tubes which did not show any visible growth was culture on MHA plate. The plate that did not show any growth was considered as MBC.

5. Phytochemical test of leaf extract

Phytochemical analysis for major phytoconstituents of torch ginger leaf extract was undertaken using standard qualitative methods to screen for the presence of alkaloids, glycosides, saponins, flavonoids, tannins, phenols, and terpenoids.

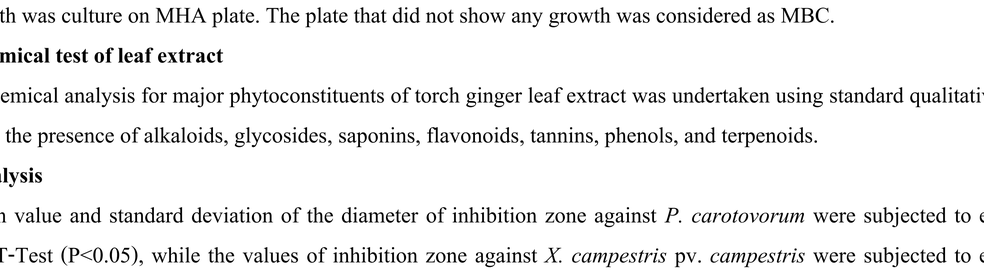
6. Data analysis

The mean value and standard deviation of the diameter of inhibition zone against *P. carotovorum* were subjected to examine by analysis of T-Test ($P<0.05$), while the values of inhibition zone against *X. campestris* pv. *campestris* were subjected to examine by analysis of variance (ANOVA) followed by Fisher's LSD test ($P<0.05$).

Results and Discussion

1. Antibacterial activity

As show in Table 1 and Figure 1, methanolic leaf extract gave the best antibacterial activity against *P. carotovorum* and *X. campestris* pv. *campestris*. Interestingly, the methanolic extract showed the higher inhibition zone than positive control. Acetone extract showed the MIC against *P. carotovorum* and *X. campestris* pv. *campestris* at concentration 12.56 and 25 mg/mL followed by methanolic extract at concentration 50 and 100 mg/mL, respectively. Moreover, ethanolic extract showed the MIC value of 200 mg/mL against *X. campestris* pv. *campestris*. All three extracts showed MBC at concentration of 400 mg/mL (Table 2). This present study found that the polarity of solvent seemed to play an important role in the antibacterial activity of the extracts (Jeyaseelan et al., 2010). Similar to previous report indicated that high polarity organic solvents such as ethanol and methanol were suitable extraction solvents for extraction of bioactive compounds from plants (Hanphakphoom et al., 2016).



Dried leaves and 50 mg/mL leaf extracts of *Etlingera elatior*

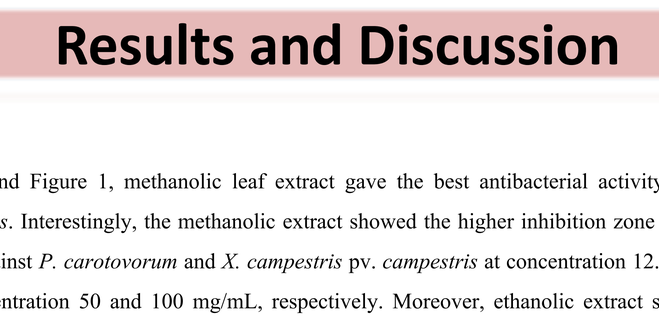


Figure 1. Antibacterial activity of methanolic leaf extract against *Pectobacterium carotovorum* (A) and *Xanthomonas campestris* pv. *campestris* (B)

Table 1. Antibacterial activity against *Pectobacterium carotovorum* and *Xanthomonas campestris* pv. *campestris* of torch ginger leaf extracts

Extracts by solvents	Diameter of inhibition zone (mm)	
	P. carotovorum	*X. campestris* pv. *campestris*
Hexane	-	-
Dichloromethane	-	-
Acetone	10.07 ± 0.87^b	10.33 ± 0.24^b
Ethanol	-	7.75 ± 0.32^c
Methanol	12.56 ± 0.22^a	12.97 ± 0.64^a
Clear	11.65 ± 0.90	11.58 ± 0.77

Note: Diameter of inhibition zones includes diameter of well. Values are represent as mean ± SD of three replicates; - represent no inhibition; In each column, different superscripts represent significant differences ($p \leq 0.05$).

Table 2. Minimum inhibition concentration (MIC) and Minimal bactericidal concentration (MBC) against *Pectobacterium carotovorum* and *Xanthomonas campestris* pv. *campestris* of torch ginger leaf extracts

Extracts by solvents	*P. carotovorum*		*X. campestris* pv. *campestris*	
	MIC	MBC	MIC	MBC
Hexane	-	-	-	-
Dichloromethane	-	-	-	-
Acetone	12.56	400.00	25.00	400.00
Ethanol	-	-	200.00	400.00
Methanol	50.00	400.00	100.00	400.00

Note: MIC and MBC were measured as mg/mL; - represent no test

2. The phytochemical constituents

The phytochemical constituents of leaf extract of torch ginger were presented in Table 3. The results showed that all extracts contained alkaloids and glycosides. Both methanolic and ethanolic extracts contained all tested components including alkaloids, glycosides, saponins, flavonoids, tannins, phenols, and terpenoids while acetone extract contained alkaloids, phenols, glycosides, and tannins. Phytochemical screening of methanolic extract of torch ginger inflorescences was reported the containing of flavonoids, terpenoids, saponins, tannins, and carbohydrates (Lachumy et al., 2010). These finding were in line with previous research that reported the inhibition activity of these substances against pathogenic bacteria (Ali et al., 2017).

Table 3. Phytochemical of hexane, dichloromethane, acetone, ethanolic and methanolic leaf extracts

Phytochemical Constituents	Torch ginger extract				
	Hexane	Dichloromethane	Acetone	Ethanol	Methanol
Alkaloids	+	+	+	+	+
Flavonoids	-	-	-	+	+
Phenols	-	+	+	+	+
Glycosides	+	+	+	+	+
Terpenoids	-	-	-	+	+
Tannins	-	+	+	+	+
Saponins	-	+	-	+	+

Note: (-): absence; (+): presence

Conclusion

The study revealed the antibacterial activity of acetone, ethanolic, and methanolic leaf extracts of *E. elatior* against *P. carotovorum* and *X. campestris* pv. *campestris*. This activity was affected by their phytochemical constituents. Phytochemical analysis indicated that acetone leaf extract contained alkaloids, glycosides, tannins, and phenols while methanolic and ethanolic leaf extract contained alkaloids, glycosides, saponins, flavonoids, tannins, phenols, and terpenoids. This plant can therefore be used as a new source for pesticide production.

Acknowledgement

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