The Value Addition of Cassava Leaves from Extracted Tannins with means of Inhibiting and Eliminating *Escherichia coli*: as an Economic Alternative for Wastewater Treatment

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ABSTRACT

This research had the main objective to add value of residue cassava leaves by extracted tannins by means of inhibiting and eliminating *Escherichia coli* in domestic wastewater treatment. The specific objective of the study aimed to investigate the efficiencies of *Escherichia coli* inhibition and elimination by extracted tannins from cassava leaves in domestic wastewater. The experiments were carried out by focusing on *Escherichia coli* in domestic wastewater from the main canteen of Nakhon Ratchasima Rajabhat University. The dried cassava leaves (Kasetsart-50) was used. The 80% acetone was used as a solvent for extraction. Analysis of *Escherichia coli* in wastewater was performed by method of the Most Probable Number (MPN). The results found that the treatment 4 (Tannin 20 mL/L) was the highest inhibition and elimination of *Escherichia coli* at all time points. The efficiency of inhibition and elimination of *Escherichia coli* in the treatment 3 (Tannin 10 mL/L) was slightly lower than that of the treatment 4 (Tannin 20 mL/L). The results also showed that all these three experiments showed a rapid increase of efficiencies in the first hour. In general, the tannin with concentration of 10 mL/L may be considered as the optimum concentrations with the reaction time of an hour. The tannin concentration of 10 mL/L should be selected for the wastewater treatment with respect to the economy reason. Economic comparison between the imported tannins and Thai domestically manufactured tannins showed that the Thai domestically manufactured tannins can save cost up to 75%. This study provided an economic alternative for wastewater treatment by using natural extracts instead of chemicals such as chorine for disinfection. As a result of that, it increased the value of the residue cassava leaves.

Keywords: Value addition, Extracted tannins, Cassava leaves, *Escherichia coli*

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is currently planted in approximately 2,766,798 acres (7,000,000 rais) of land in 48 provinces in Thailand, which produce over 20 million tons of cassava roots [1]. Cassava or tapioca is considered one of the most Thailand's economic crops, especially, in the northeasthern part of Thanland. The cassava is used in many industries including tapioca flour, tapioca pellets and animal food production. A large number of its leaves and stems which are left over materials will be later used as many purposes such as animal food. It can also be used in the production of organic fertilizer and ethanol. The further utilizations increase value of all parts of cassava. In the present time, the substance (tannins) in cassava leaves is increasingly extracted and investigated for further utilization that tannin extraction has been gradually examined in inhibiting and eliminating microorganisms [2-4].

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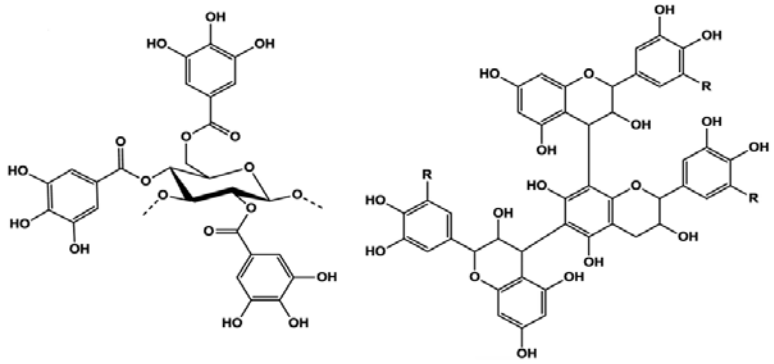
Tannins ($C_{75}H_{52}O_{46}$), also called Tannic acid, are phenolic compounds as a large and complex molecule. Their main characteristics are that they bind and precipitate proteins, a weak acid and astringent taste. Tannins are found in the roots, wood, bark, leaves and fruit of many plants, for examples, oak, chesnut, grapes (both skins and seeds), tea leaves, jatropha leaves, nuts, coffee, corn, mangosteen husk, banana husk, and rambutan husk. Tannin is divided in two types. One is hydrolysable tannin (Gallotannin) that it is found in leaves and sheath. The other is condensed tannin (Proanthocyanidin) found in the bark and cores of the plants [5]. The molecule of tannin structures are shown in Figure 1 [6]. Tannins are widely used in tanning leather industry and used as dyeing fabric, making ink and antimicrobial agents.



(a) (b)

Fig. 1 Tannin structures: (a) hydrolysable tannin and (b) condensed tannin [6]

Escherichia coli (*E. coli)* are gram negative bacteria (GNB), rod in shape, facultative anaerobic bacteria, non-sporulated bacteria. It is classified in the family of Enterobacteriaceae which resides in an animal gastrointestinal tract. Enterobacteriaceae includes *Eschericia, Klebsiella, Proteus, Enterobacter, Serratia, Citrobacter, Morganella* etc. *Escherichia coli* are one type of coliform bacteria which is considered as an indicator of fecal contamination in water. Generally, it is found in the lower part of animal and human intestine. Thus, this bacterium can cause some diseases such as diarrhea both in children and adults who consume contaminated foods and water with *Escherichia coli* [7-8].

Therefore, this research had the main objective to add value of residue cassava leaves by extracted tannins with means of inhibiting and eliminating *Escherichia coli* in domestic wastewater treatment. The specific objectives of the study aimed to investigate the efficiencies of *Escherichia coli* inhibition and elimination by extracted tannins from cassava leaves in wastewater from the main canteen of Nakhon Ratchasima Rajabhat University in order to add value to cassava leaves. Analysis of *Escherichia coli* in wastewater was performed by method of the Most Probable Number (MPN). Dried leaves of cassava were used for this study by using solvent of 80% acetone for extraction.

2. METHODOLOGY

This research was to study the efficiency of extracted tannins from cassava leaves to inhibiting and eliminating *Escherichia coli* in wastewater from the main canteen of Nakhon Ratchasima Rajabhat University. The dried cassava leaves (Kasetsart-50 or K-50) were used. The 80% acetone was used as a solvent for extraction. Analysis of *Escherichia coli* in wastewater was performed by method of the Most Probable Number (MPN).

2.1 The simple extraction of tannin from cassava leaves

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The dried cassava leaves of K-50 were extracted by 80% Acetone. In the preliminary study, it has been reported that the dried cassava leaves of K-50 which was extracted by 80% acetone provided the optimum yield according to Waraporn K. and Rachain K. (2015) [9]. The ratio between the cassava leaves to solvents was 1:20 at 3 hours extraction time with shaking at 150 rpm [10]. The tannin concentrations were determined by UV/VIS Spectrophotometer (SHIMADZU UV-1601) at 762 nm of wavelength in terms of mg/kg. Consequently, all the tannin extracts were used in the next experiment as explained below.

2.2 The efficiency study of tannin extract to inhibiting and eliminating *Escherichia coli* in wastewater

Efficiency of extracted tannins from cassava leaves to inhibiting and eliminating *Escherichia coli* in wastewater from the main canteen of Nakhon Ratchasima Rajabhat University. The experiments were conducted by the cyclider wastwater treatment reactor as shown in Figure 2 [11].

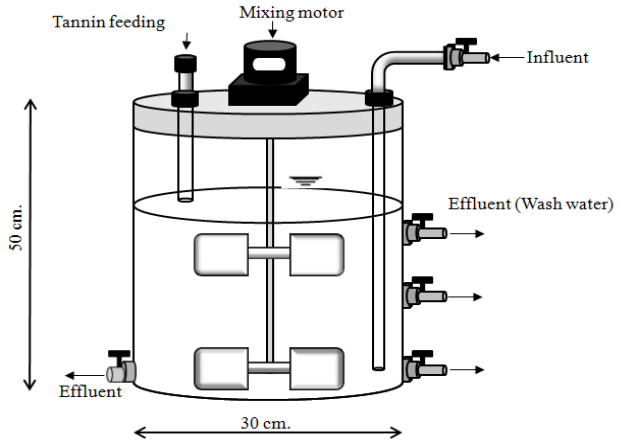

Fig. 2 Wastewater treatment reactor
(Modified from Rachain K. And Waraporn K., 2013) [11]

The experiments were divided into 4 treatments on the below lists:
Treatment 1 Control: Five litres of wastewater without tannins
Treatment 2 Tannin 2 mL/L: Five litres of wastewater with the tannins of 10 mL
Treatment 3 Tannin 10 mL/L: Five litres of wastewater with the tannins of 50 mL
Treatment 4 Tannin 20 mL/L: Five litres of wastewater with the tannins of 100 mL

All experiments were operated for a designated period of time in wastewater treatment reactor. The reaction times after adding tannin were varied at 0, 1, 2, 4 and 8 hours with 150 rpm mixing. Subsequently, the analysis of *Escherichia coli* was carried out by method of Most Probable Number [12-13] in terms of the MPN/100mL. This method consists of two parts, namely the presumtive test and the confirmed test.

In the presumptive test, a series of test tubes of lactose broth were inoculated with varied amounts of wastewater. The gas was produced and observed in Duran tube that the amount of gas was subject to the lactose fermenting bacteria [14]. Those bacteria presumably belonged to coliform bacteria. The quantity of coliform bacteria was directly correlated to the volume of rising gas after the incubation for a required period of time at 35° C 24 hours [10-13]. For the confirmed test, the test was carried out to confirm that the coliform bacteria from the presumptive test were *Escherichia coli*. The positive samples from the presumptive test were further cultured on Eosin Methylene Blue (EMB) agar by using streak plate technique. Later, the plates were incubated at 35° C for 24 hours

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and the number of colonies was counted. The colonies of *Escherichia coli* were recognized by their color which was described as dark green or black with typical greenish metallic sheen. The results were expressed as MPN/100 mL, means, standard deviations (SD) and percentages of the removal efficiency. Each experiment was performed with three replications.

3. RESULTS AND DISCUSSION

3.1 The efficiency study of tannin extract to inhibiting and eliminating *Escherichia coli* in wastewater

The results of 4 treatments which varied the concentration of tannins (0, 2, 10 and 20 mL/L Tannins) and removal reaction times (0, 1, 2, 4 and 8 hours) for studying the removal efficiency of Tannin from cassava leaves to inhibiting and eliminating *Escherichia coli* in wastewater. It was analyzed by method of Most Probable Number [12-13] in terms of the MPN/100mL. This method consists of two parts, namely the presumtive test and the confirmed test as shown below.

3.1.1 The results of the presumptive test

The results of the presumptive test of Most Probable Number were showed in Table 1 and Figure 3. The results demonstrated the quantity of *Escherichia coli* in 4 treatments in respond to the observed gas (+ = positive, - = negative) in relation to varing connentration of Tannin and reaction times.

Table 1 Analysis in quantity of *Escherichia coli* in wastewater sample by the presumptive test of Most Probable Number

Treatments	Reaction times	Amount of water			MPN Index	MPN/100 ml
		10 ml	1 ml	0.1 ml		
Treatment 1	0 hr.	+ + +	+ + +	+ + +	>1,100	>11,000
Control	1 hr.	+ + +	+ + +	+ + +	>1,100	>11,000
	2 hrs.	+ + +	+ + +	+ + +	>1,100	>11,000
	4 hrs.	+ + +	+ + +	+ + +	>1,100	>11,000
	8 hrs.	+ + +	+ + +	+ + +	>1,100	>11,000
Treatment 2	0 hr.	+ + +	+ + +	+ + +	>1,100	>11,000
Tannin 2 mL/L	1 hr.	+ + +	- + +	+ - +	210	2,100
	2 hrs.	+ + +	+ - -	+ + +	160	1,600
	4 hrs.	+ + +	+ - -	+ - +	120	1,200
	8 hrs.	+ + -	- + +	+ + +	42	420
Treatment 3	0 hr.	+ + +	+ + +	+ + +	>1,100	>11,000
Tannin 10 mL/L	1 hr.	+ - +	- - +	- - +	20	200
	2 hrs.	+ - -	+ - +	+ + -	20	200
	4 hrs.	+ - -	+ + +	- - -	16	160

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Treatments	Reaction times	Amount of water			MPN Index	MPN/100 ml
		10 ml	1 ml	0.1 ml		
	8 hrs.	- + -	+ - -	+ - -	11	110
Treatment 4	0 hr.	+ + +	+ + +	+ + +	>1,100	>11,000
Tannin 20 mL/L	1 hr.	- + -	- + +	- + +	20	200
	2 hrs.	- + -	- + +	+ - -	15	150
	4 hrs.	+ - +	- + -	- - -	15	150
	8 hrs.	+ - -	+ - -	- - -	7.3	73

Note + Positive test (Gas observed at 24 hour)
- Negative test (No gas at 24 hour)

Table 1 and Figure 3 showed that the treatment 4 demonstrated the minimal number of remaining *Escherichia coli* at all time points of incubation with tannins. Accordingly, the quantities of remaining *Escherichia coli* in the treatment 3 were found slightly higher than those of the treatment 4. The treatment 2 showed that the quantities of remaining *Escherichia coli* were moderately higher than those of the treatment 3 and 4. In addition, all these three treatments showed a rapid decrease of *Escherichia coli* in the first hour of incubation time with tannins.

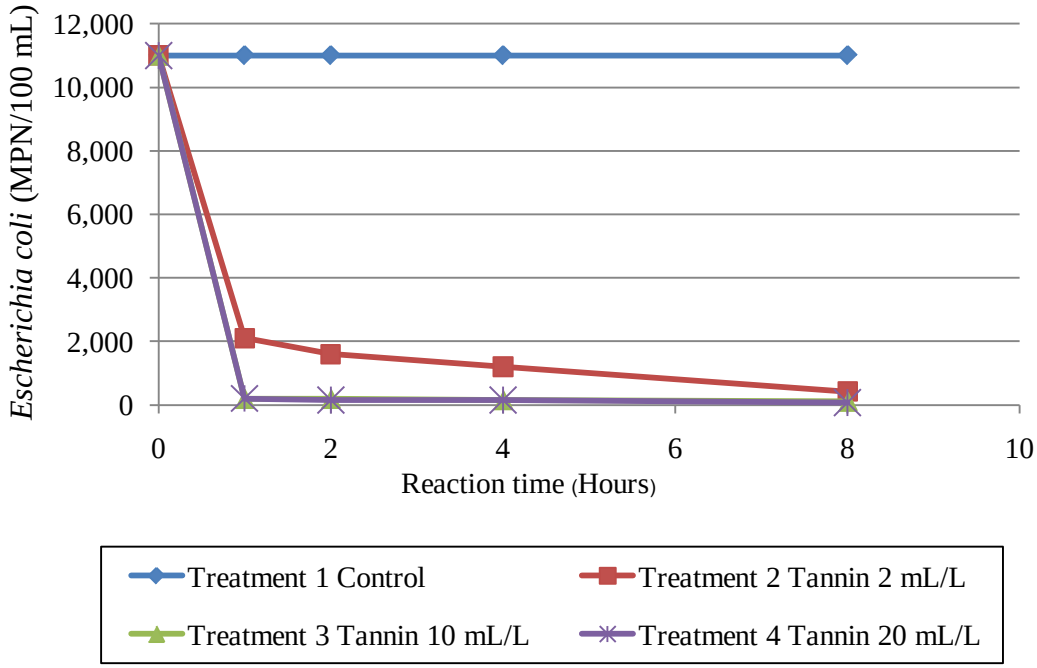

Fig. 3 Quantity (MPN/100 ml) of coliform bacteria in wastewater sample by the presumptive test of Most Probable Number

Percentages of the efficiency removal in inhibiting and eliminating coliform bacteria in wastewater sample were showed in Figure 4. The treatment 4 demonstrated the most efficiency in inhibiting and eliminating *Escherichia coli* in wastewater up to 99.34 at the time point of 8 hour. However, the percentages of an hour time point was 98.18 which was slightly less than those of 2, 4, 8 hour time points. The treatment 3 showed the similar results to the treatment 4. The treatment 2 showed that the percentages of efficiency were moderately less than those of the treatment 3 and 4. This indicated that the tannin with concentration of either 10 or 20 mL/L was probably considered

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as the optimum concentration with the incubation time of an hour. In some circumstances, the tannin concentration of 10 mL/L should be selected for the wastewater treatment with respect to the economy reason.

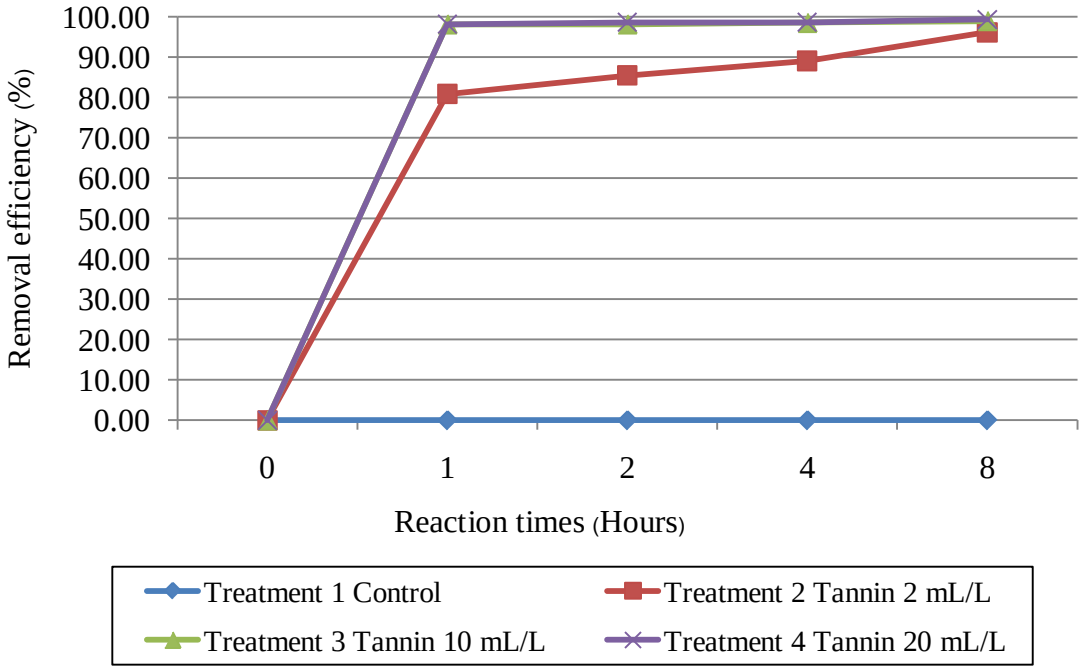

Fig. 4 Efficiency in inhibiting and eliminating *Escherichia coli* in wastewater sample by the presumptive test of Most Probable Number

3.1.2 The results of confirmed test

The results of the confirmed test of Most Probable Number were showed in Figure 4.

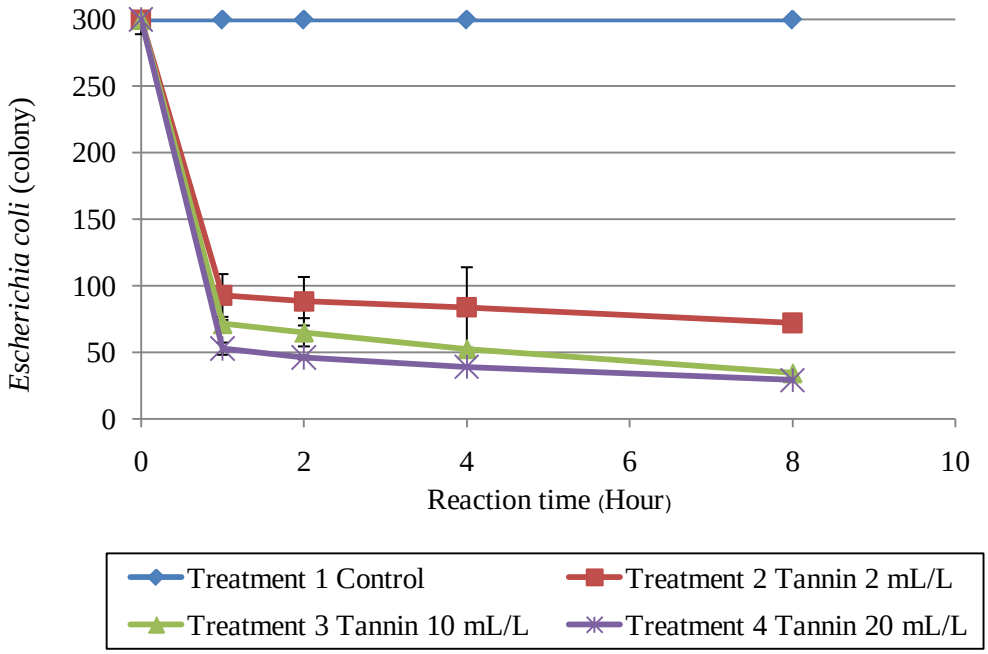

Fig. 4 Number of *Escherichia coli* colonies in streak plates by the confirmed test of Most Probable Number

Figure 4 showed the number of *Escherichia coli* colonies on the streak plates after treatment with the extract tannins from cassava leaves. The treatment 4 demonstrated the minimum of colonies

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at all time points. It was decreased down to 29 at the time point of 8 hours. The treatment 2 and 3 were found that the numbers of colonies were higher than those of the treatment 4. In addition, all these three treatments showed a rapid decrease of *Escherichia coli* number in the first hour.

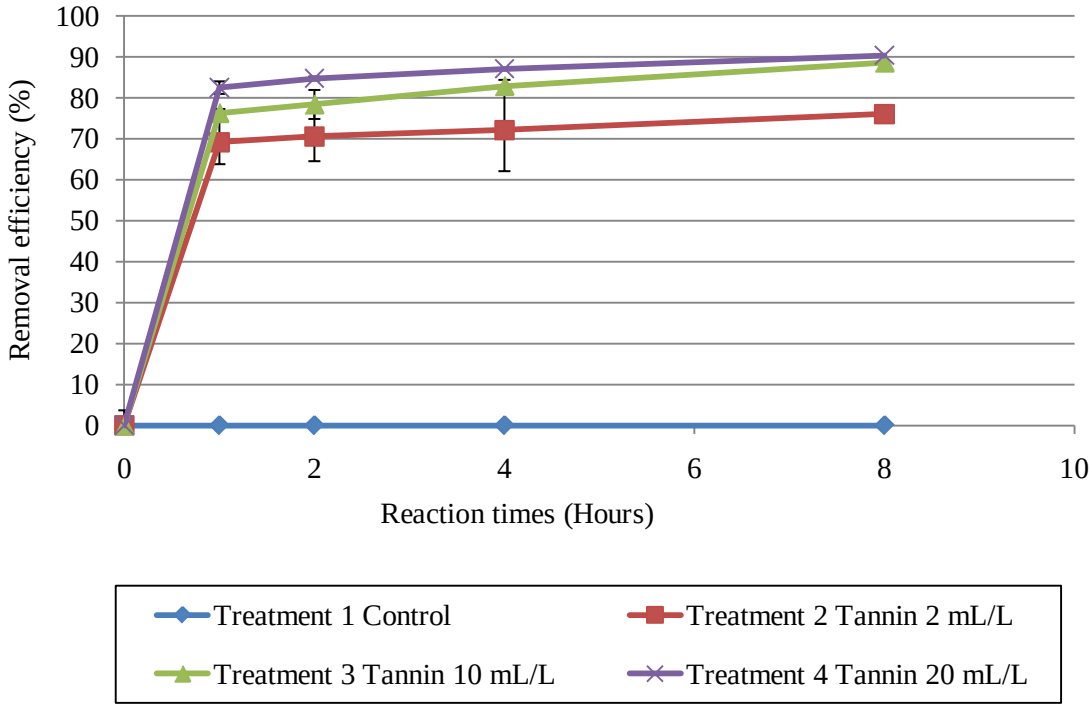

Fig. 5 Efficiency in inhibiting and eliminating *Escherichia coli* in streak plates by the confirmed test of Most Probable Number

Figure 5 showed efficiencies in inhibiting and eliminating *Escherichia coli* in terms of the number of colonies on the streak plates after treatment with the extract tannins from cassava leaves. The treatment 4 demonstrated the maximun of efficiency at all time points. It was increased up to 99.3% at the time point of 8 hours. The treatment 2 and 3 were found that the efficiencies were lower than those of the treatment 4. In addition, all these three treatments showed a rapid increase of efficiencies in the first hour. As previously stated in the presumptive test, the confirmed test indicated that the tannin with concentration of 10 mL/L may be considered as the optimum concentrations with the incubation time of an hour. In certain circumstances, the tannin concentration of 10 mL/L should be selected for the wastewater treatment with respect to the economy reason.

3.2 Economic comparison between the imported tannins and Thailand's domestic manufactured tannins

Table 1 Economi comparison between the imported tannins and Thailand's domestic manufactured tannins

Imported tannins	Thailand's domestic manufactured tannins	Cost saving	Percentage of savings
1,200 THB/liter (30 USD/liter)	300 THB/liter (7.5 USD/liter)	900 THB/liter (22.5 USD/liter)	75%

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In Thailand, the tannins are mostly imported from abroad especially in Brazil and New Zealand [15]. The price of imported tannins are rather expensive. It is highly likely to increase 1,200 THB/liter (30 USD/liter). However, if it is domestically manufactured tannins which the price could be only 300-500 THB/liter (7.5 USD/liter) [14]. Thus, this data showed that domestically manufactured tannins were 900 THB/liter (22.5 USD/liter) cost saving or it can save cost up to 75%. Moreover, the tannins in Thailand are more likely to be extracted from cassava leaves and certainly increase the value to cassava leaves. As a result of this, it can generate more income to the farmers. It has been known that dried cassava leaves can be sold to the industry for 5-10 THB/kg [15]. This study provided an economic alternative for wastewater treatment by using natural extracts instead of chemicals such as chorine for disinfection or antimicrobial effects according to Cee K lim *et al*. (2013) and Alexanda H smith *et al*. (2003) [16-17]. Therefore, it indicated the value-added activity of the residue cassava leaves.

4. CONCLUSION

The current study revealed the efficiencies of *Escherichia coli* inhibition and elimination by extracted tannin from cassava leaves. The experiments were carried out by focusing on *Escherichia coli* in wastewater from the main canteen of Nakhon Ratchasima Rajabhat University. The results found that the treatment 4 (20 mL/L Tannin) was the highest inhibition and elimination of *Escherichia coli* at all time points. The efficiency of inhibition and elimination of *Escherichia coli* in the treatment 3 was slightly lower than that of the treatment 4. Accordingly, the results showed that all three experiments showed a rapid increase of efficiencies in the first hour. Generally, the tannin with concentration of 10 mL/L may be considered as the optimum concentrations with the incubation time of an hour. In addition to this, the tannin concentration of 10 mL/L should be selected for the wastewater treatment with respect to the economy reason. This study demonstrated that the further utilization of cassava leaves was more possible by means of inhibiting and eliminating *Escherichia coli* for the value-added activity to the cassava leaf residue. Therefore, the tannin from the cassava leaves could be one of alternatives that it can be used to treat wastewater by means of inhibiting and eliminating *Escherichia coli*. Economic comparison between the imported tannins and Thailand's domestic manufactured tannins showed that the Thailand's domestic manufactured tannins were a 75 percent reduction in cost.

5. ACKNOWLEDGEMENTS

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