The Approach for Utilization of Extracted Tannins from Cassava Leaves as Bactericide: Preliminary Study

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ABSTRACT

The study involving extracted tannins from cassava leaves aimed to investigate the efficiency of the extracted tannins from cassava leaves in terms of inhibition and elimination of *Escherichia coli* in preliminary test. The results showed that the dried K-50 cassava leaves that extracted by 80% acetone provided a maximum amount of the extracted tannins (398.32±3.20 mg/kg). With regard to the efficiency of extraction, it was found that the dried leaves were higher than the fresh leaves. The results also demonstrated that 80% acetone showed higher efficiency than 80% ethanol and distilled water, respectively. At temperature 50 ºC, it showed higher efficiency than that of the temperature 25 ºC. The study of the efficiency of *Escherichia coli* inhibition and elimination by extracted tannins from cassava leaves with solvent evaporation exhibited that the highest inhibition and elimination of *Escherichia coli* was found in the experiment with the dried K-50 cassava leaves extracted by 80% acetone. This indicated that extracted tannins induced the *Escherichia coli* inhibition and elimination. This research can be concluded that the extracted tannins from cassava leaves can, at least in part, inhibit and eliminate *Escherichia coli*. As a result of this, there was a possibility to increase the more utilization and applications of cassava leaves as well as its added value in the near future.

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

1. INTRODUCTION

*Escherichia coli (*commonly abbreviated *E. coli)* bacteria normally live in the lower part of animal and human intestine. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract both in children and adults [1-2]. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contacts with animals or persons [3]. *E. coli* are Gram-negative bacteria (which do not retain crystal violet dye), facultative anaerobic (which makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or anaerobic respiration if oxygen is absent) and nonsporulating [4]. Cells are typically rod-shaped, and are about 2.0 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3 [4]. It belongs to the family of Enterobacteriaceae which resides in an animal gastrointestinal tract. Enterobacteriaceae includes *Eschericia, Klebsiella, Proteus, Enterobacter, Serratia, Citrobacter, Morganella* etc. *E. coli* is one type of coliform bacteria which is considered as an indicator of fecal contamination in water. This bacterium can cause diarrhea who consume contaminated foods and water with *E. coli*. [1-2]

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Cassava (*Manihot esculenta* Crantz), also called tapioca, is one of Thailand's industrial crops, especially, in Nakhon Ratchasima province. Cassava's yield is used in various industrial proposes including tapioca flour, tapioca pellets and animal food production. The leaves and stems of cassava which are left over materials will be later used as animal food production. Moreover, it can also be utilized in the production of organic fertilizer and ethanol. These utilizations increase value of all parts of cassava. Nowadays, the substance, tannins, in cassava leaves is extracted and increasingly studied for further benefits. This also is value added. Tannin extraction has been investigated and increasingly used as pesticides to eliminate and minimize the spread of microorganisms such as bacteria.

Tannins ($C_{75}H_{52}O_{46}$) are large and complex molecules. Tannins are weak acid and astringent taste. Tannins can be found in various parts of some plants, for examples, grape seeds, tea leaves, coffee, corn, jatropha leaves, nuts, banana husk, rambutan husk and mangosteen husk. Tannins are divided in two types. One is the condensed tannin (Proanthocyanidin) that is found in the bark and cores of the plants. The other is the hydrolysable tannin that can be found in leaves and sheath [5]. The basic structure of tannin is shown in Figure 1 [6].

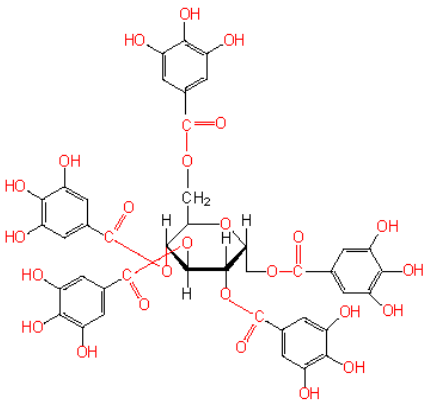

Fig. 1 Basic structure of Tannin [6]

Nowadays, the tannins are mainly imported from abroad, especially, Brazil and New Zealand [7]. The price of imported tannins is up to 1,200 baht per liter. It is also able to be manufactured in Thailand. The price could be only 500 baht per liter [7]. Therefore, this study aimed to investigate the efficiency of the extracted tannins from cassava leaves in terms of inhibition and elimination of *E. coli* in order to add value to cassava leaves. Two strains of cassava were studied with both dried and fresh leaves. Three types of solvents using for tannin extraction were 80% acetone, 80% ethanol and distilled water under two different temperatures (25ºC and 50ºC).

2. METHODOLOGY

This present study was divided into 2 parts. The first part was the study of simple tannin extraction from cassava leaves in terms of tannin concentration (mg/mL) under various conditions. The second part was the efficiency study of extracted tannins from cassava leaves to inhibiting and eliminating *E. coli*.

2.1 The simple extraction of tannins from cassava leaves

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Two strains of cassava leaves, namely Kasetsart-50 (K-50) and Rayong-90 (R-90), were extracted in forms of both dried and fresh leaves. Three types of solvents were used in tannin extraction. There were 80% acetone, 80% ethanol and distilled water. Moreover, the different temperatures in tannin extraction, 25ºC and 50ºC were used in this study. The ratio between the cassava leaves to solvents was 1:20 at 3 hours extraction time with shaking at 150 rpm as described previously by Wongsuksin K., 2013 [8]. The tannin concentrations were determined by UV/VIS Spectrophotometer (SHIMADZU UV-1601) at 762 nm of wavelength in terms of mg/kg. All the extracted tannins were later used in the next experiments as explained below.

2.2 The efficiency study of extracted tannins from cassava leaves to inhibiting and eliminating Escherichia coli

E. coli (TISTR 780) were cultured on Eosin Methylene Blue Agar (EMB) and incubated at 37ºC for 24 hours and used in this study. A single colony with dark green or black with typical greenish metallic sheen was selected and transferred into food Nutrient Broth (NB) in test tubes. Later, the *E. coli* was incubated for 24 hours before it was used for further inhabitation testing.

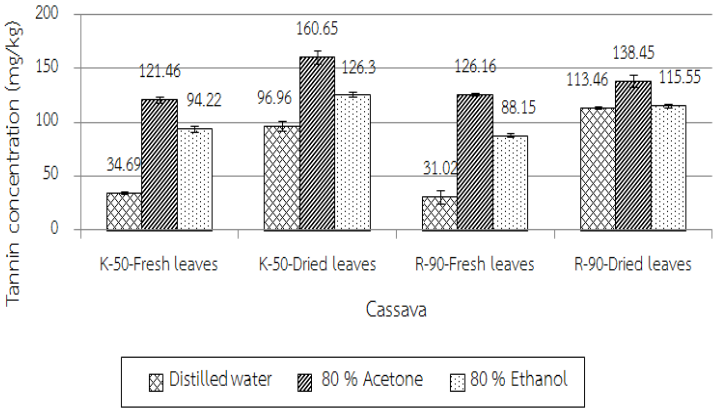
The turbidimetric method was used to determine quantity of *E. coli* by measuring optical density (OD) with the spectrophotometer at 600 nm of wavelength. This is due to the fact that the turbidity or OD is directly proportional to the density or a number of cells. Then, the density of *E. coli* (CFU/ml) was determined by using the standard curve that showed the relationship between OD and the density (CFU/ml).

For the inhabitation tests, the selected colony of *E. coli* was added with one of those extracted tannins as described above. The sample was incubated for 24-48 hours before measurement for cell density by the turbidimetric method. The data were expressed in terms of means and standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 The simple extraction of tannins from cassava leave

For the simple extraction of tannins from cassava leaves, two strains of cassava were used in this study in forms of both fresh and dry leaves. Three types of solvents were used, namely, 80% acetone, 80% ethanol and distilled water. The results were explained below



(a)

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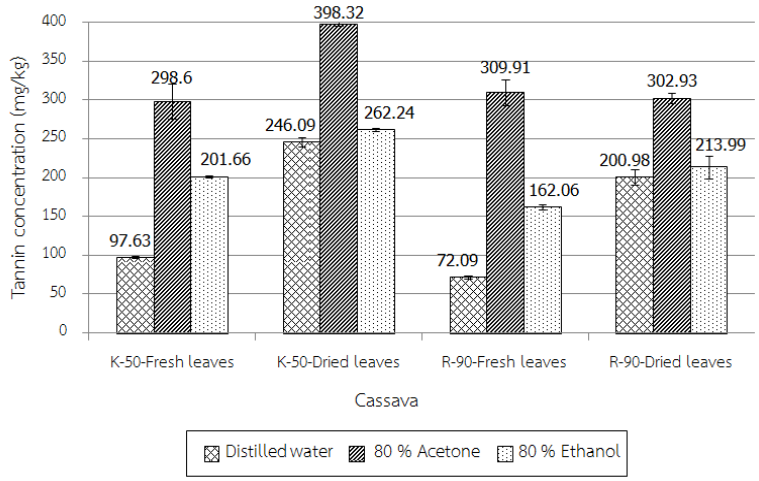

(b)

Fig. 2 Concentration of Tannins from cassava leaf extraction at (a) 25 ºC and (b) 50 ºC

The figure 2 (a) showed the concentration of extracted tannins from cassava leaves at 25 °C. It found that the dried cassava leaves of K-50 which was extracted by 80% acetone provided the highest concentration of tannins (160.65±6.58 mg/kg). The dried cassava leaves of R-90 which was also extracted by 80% acetone provided the tannin concentration of 138.45±5.26 mg/kg. Moreover, the lowest tannin concentration was of the fresh cassava leaves of R-90 which was extracted by distilled water (31.02±6.03 mg/kg). The results demonstrated that the tannin concentrations of the dried leaves were higher than those of fresh leaves regardless of extract solvents and cassava strains. Moreover, 80% acetone was the best solvent which provided the highest tannin concentration, followed by 80% ethanol and distilled water, respectively.

In the experiments with the temperature of 50 ºC that was shown in the figure 2 (b), the results were found that the highest concentration (398.32±3.20 mg/kg) of tannins was from the dried leaves of K-50 with 80% acetone as a solvent. It was followed by fresh leaves of R-90 with 80% acetone as a solvent providing the tannin concentration of 309.91±16.19 mg/kg. The fresh leaves of R-90 extracted by distilled water showed the lowest tannin concentration (72.09±1.78 mg/kg). It was also found that the tannin concentrations from the dried leaves were higher than those of fresh leaves in all experiments regardless of extract solvents and cassava strains. Moreover, 80% acetone was the best solvent which found highest concentration of tannins followed by 80% ethanol and distilled water, respectively. These results were similar to the experiment at 25 ºC.

In comparison of the experiments between 25 and 50 °C, it showed the efficiency of tannin extraction at 50 °C was higher than 25 °C in all experiments when compared with the same solvents or the strains. Moreover, 80% acetone demonstrated highest efficiency at both 25 and 50 °C, followed by 80% ethanol and water, respectively.

3.2 The efficiency of extracted tannins from cassava leaves to inhibiting and eliminating *Escherichia coli*

Efficiency of extracted tannins from cassava leaves to inhibiting and eliminating *E. coli* was shown in Figure 3.

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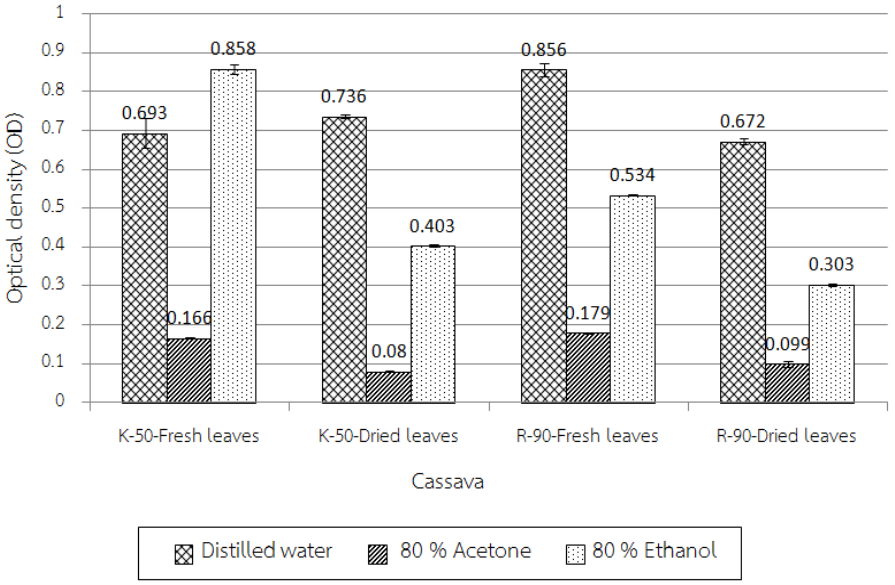
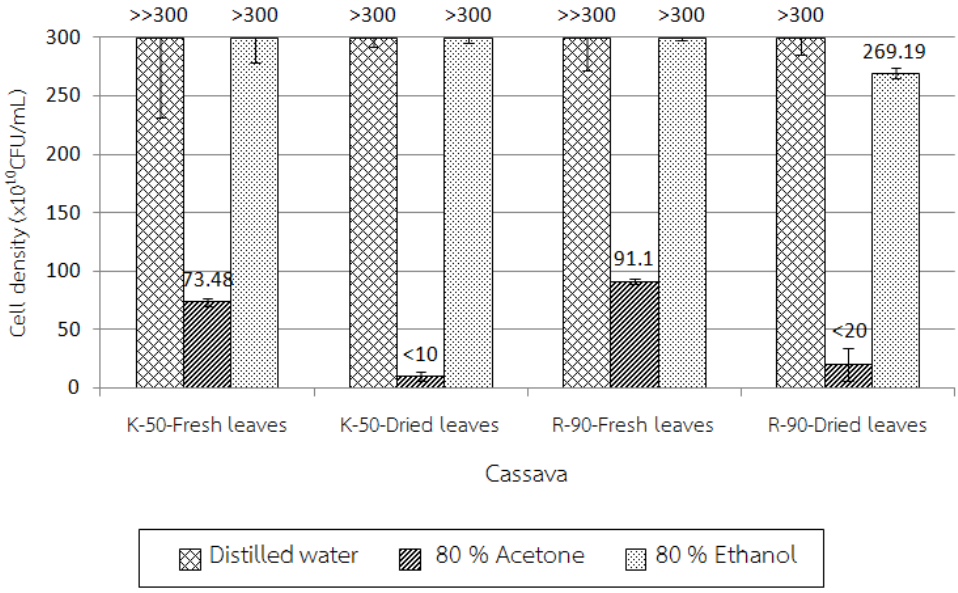


(a)



(b)

Fig.3 (a) Optical density (OD) and (b) Cell density (x10^{10}CFU/mL) of *Escherichia coli*

Figure 3(a) and (b) showed the turbidity or optical density (OD) and cell density (×10^{10}CFU/mL) in inhibiting and eliminating *E. coli* by extracted tannins from cassava leaves in different solvents. The results showed that the extracted tannins from dried K-50 cassava leaves using acetone as solvent demonstrated the highest effective inhibition to *E. coli* due to that the turbidity or optical density (OD) and cell density (×10^{10}CFU/mL) was the minimum with 0.08 OD and <10 x 10^{10}CFU/mL. Overall, the inhibition and elimination of *E. coli* by extracted tannins from cassava leaves can be described as follows: extracted tannins from 80% acetone as a solvent in extraction was the most effective, followed by 80% ethanol and distilled water, respectively. In addition, Figures 3(a) and (b) illustrate extracted tannins of dried cassava leaves were higher effective in inhibition and elimination than fresh cassava leaves. Moreover, it was found that K-50 cassava leaves showed higher inhibition and elimination than that of R- 90 cassava leaves.

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4. CONCLUSION

The present study showed that the extracted tannins from dried K- 50 cassava leaves using acetone as solvent provided the highest effective inhibition and elimination in *E. coli* growth. The extraction of tannins from cassava leaves between 25 and 50 ℃ can be concluded that the efficiency of tannin extraction at 50 ℃ was higher than 25 ℃. Moreover, the experiments with 80% acetone provided highest efficiency at both 25 and 50 ℃, followed by 80% ethanol and water, respectively. Dried cassava leaves demonstrated higher efficiency of tannin extraction than that of fresh cassava leaves in the experiments of both strains of cassava. Moreover, it showed that K-50 cassava leaves provided higher inhibition and elimination than that of R-90 cassava leaves. This present study indicated that extracted tannins from cassava at least partly induced the *E* inhibition and elimination due to the reverse relationship between quantitation of tannins and the *E. coli*. As a result of this, it is possible to increase value of cassava leaves as well as its utilization. However, the tannins from the cassava leaves should be further investigated for possibility of implementation to treat wastewater by means of inhibiting and eliminating *E. coli*.

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