Efficacy of Tea Leaf Extracts for Inhibition of Pathogenic Enteric Bacteria and Anti-free Radicals Activity

ประสิทธิภาพของสารสกัดใบชาสำหรับการยับยั้งแบคทีเรียก่อโรคในระบบทางเดินอาหาร และฤทธิ์ต้านอนุมูลอิสระ

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ABSTRACT

Tea is one of the most popular beverages. Drinking tea has health benefits since tea leaves contain polyphenol, which has many properties to protect and curing diseases. Thus, objectives of this research were to investigate the inhibition of pathogenic enteric bacteria and anti-free radical activities of tea leaf extract. The result showed that green tea leaf extract after extraction for 3 hours showed the highest anti-bacterial activity against pathogenic enteric bacteria. Moreover, anti-free radical activity of green tea extracts was greater than oolong tea and black tea extracts with 50% inhibitory concentration of 8 0.38±1.40 mg GAE/g green tea leaf extract. The highest phenolic and flavonoid content in green tea leaf extract after extraction for 3 hours was observed by 12.86 ± 0.11 mg GAE/ g extract and 105.42 ± 2.00 mg quercetin/g extract, respectively.

บทคัดย่อ

ชาเป็นเครื่องดื่มที่ได้รับความนิยมเป็นอย่างมาก การดื่มชานั้นมีประโยชน์ต่อสุขภาพ เนื่องจากใบชามีสารพอลิฟีนอล ซึ่งมีคุณสมบัติหลายด้านในการป้องกันและรักษาโรค ดังนั้นวัตถุประสงค์การวิจัยครั้งนี้ เพื่อศึกษาการยับยั้งแบคทีเรียก่อโรคในระบบทางเดินอาหารและฤทธิ์ต้านอนุมูลอิสระของสารสกัดจากใบชา โดยผลการวิจัยพบว่าสารสกัดใบชาเขียวจากการสกัด 3 ชั่วโมง สามารถยับยั้งเชื้อทดสอบได้ทุกชนิด นอกจากนี้สารสกัดชาเขียวมีฤทธิ์ต้านอนุมูลอิสระที่ดี โดยพบว่าค่าความเข้มข้นที่สามารถยับยั้งอนุมูลอิสระได้ 50% เท่ากับ 80.38±1.40 mg GAE/กรัมสารสกัดใบชาเขียว และพบว่าปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์ในสารสกัดใบชาเขียวจากการสกัด 3 ชั่วโมงมีปริมาณมากที่สุดเท่ากับ 12.86±0.11 mg GAE/กรัมสารสกัด และ 105.42 ±2.00 mg quercetin/กรัมสารสกัด ตามลำดับ

Key Words: Tea Leaf extract, Pathogenic Enteric Bacteria, Anti-free radical activities

คำสำคัญ: สารสกัดใบชา แบคทีเรียก่อโรคในระบบทางเดินอาหาร ฤทธิ์ต้านอนุมูลอิสระ

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Introduction

Nowadays, infection of pathogenic enteric bacteria is the most problem in human, which cause a major public health in Thailand. Enteric bacteria are endemic in tropical countries. The infection can be transmitted by contaminated food or drink. Most pathogenic enteric bacteria such as *Salmonella* and *Escherichia coli* are members of *Enterobacteriaceae*. Normally, *E. coli* is found as normal flora in intestinal human and other mammals. However, the bacteria become opportunistic pathogen and cause disease when the immune system is impaired. The infection of *Salmonella* spp. and *Shigella* spp. cause diarrhea. Some strains of *E. coli* such as enteropathogenic *E. coli* (EPEC) can cause gastroenteritis in humans. (Clements et al., 2012). The bacteria were isolated from specimens and it could be found to be the cause of the disease about 70-80 percent (Qadri et al., 2005).

Natural products from various sources such as plants, animals and microorganisms have been used as a source and provide active ingredients for the development of drugs (Ferrazzano et al., 2011). People consume plants for their health benefits or use in folklore medicine as many plants are potential source of bioactive compounds, which have many biological activities such as antioxidant, anti-inflammation, anticancer and antimicrobial activities. (Scalbert et al., 2005) Moreover, plant extracts have been widely used for food, cosmetic, pharmaceutical industries, and remedies for treatment of human diseases. The use of plant extracts is an alternative choice in recent year.

Tea is the most popular beverage from leaf extract. The important constituents of tea leaves are polyphenols. Fresh tea leaves are rich in flavanols, which are known as catechins. Epigallocatechingallate (EGCG) is found to be the most abundant catechins in tea leaves. Regarding antimicrobial activity of tea, green tea catechins are responsible for antibacterial activity. Catechins are present 30–40% of the dry weight of fresh green tea leaves (Almajano et al., 2008). Moreover, catechin serves as useful compound for treatment of infection (Taylor et al., 2002).

We reported here the findings that tea extract showed antibacterial activity against various strains of pathogenic enteric bacteria. The anti-free radicals activity, the total phenolic compound and flavonoid content of tea leaf extracts were also shown.

Objective of the study

The aim of this study was to investigate an efficacy of extract from tea leaves on inhibition of pathogenic enteric bacteria and anti-free radical scavenging activity. Total phenolic compound and flavonoid content were also determined.

Methodology

Preparation of Tea Leaf Extracts

Different types of tea including, green tea, oolong tea, and black tea were used in this study. These tea leaves were extracted with water with the ratio of material and solvent was 1:10. The samples were divided 2 groups.

Group 1: The tea leaves were soaked in water and incubated in water bath at 45° C for 3 hours. The extraction was performed twice.

Group 2: The tea leaves were soaked in water and incubated in water bath at 45° C for 1 hour. The extraction was performed twice.

The aqueous extracts were filtered using Whatman filter No.1 and concentrated by rotator evaporator. The extracts were lyophilized and stored at -20°C. The crude extracts were dissolved to a concentration of 500 mg/ml by sterile distilled water.

Antibacterial susceptibility testing by agar disc diffusion assay

Antibacterial susceptibility testing was performed using agar disc diffusion assay (Bauer et al., 1966). The tea leaf extracts were tested against *Escherichia coli*, *Salmonella typhimurium, Shigella dysenteriae, Staphylococcus aureus* and *Vibrio cholerea*. The bacteria were suspended in Mueller Hinton broth (MHB) to give $1.5x10^8$ colony forming units/ml (McFarland No.0.5) and swabbed over Mueller Hinton agar (MHA) surface, the inoculum was allowed to dry. The paper discs were soaked in the tea leaf extracts (500 mg/ml) and placed at different areas on the surface of the agar. The agar plates were incubated at 37°C for 24 hours. After 24 hours, antibacterial activity of the extracts against the tested bacteria was observed from zone of bacterial growth inhibition.

DPPH radical scavenging activity

The scavenging activity on diphenylpicrylhydrazyl (DPPH) radical was tested (Devi et al., 2008). Tea leaf extracts (500 mg/ml) were mixed with 1,500 µl of DPPH (Sigma-Aldrich) solution (0.1mM in methanol). After 20 minutes of incubation in the dark at room temperature, an absorbance at 517 nm was recorded. The control sample blank was prepared using methanol instead of tea leaf extracts. Standard solution of gallic acid (0.001-0.01 mg/ml) was prepared in methanol. The radical-scavenging activities of the samples were expressed as percentage of inhibition and calculated using $[(A_0–A_1)/A_0]$ x 100. A_0 was the absorbance of the methanol control and A_1 was the absorbance of the samples. The IC_{50} value denoted the effective concentration of sample that used to reduce DPPH radicals by 50 % . The DPPH radical scavenging activity of the sample was calculated by [IC_{50} of gallic acid/IC_{50} of sample] x 1000 and expressed as mg gallic acid equivalent (GAE) per 1 g of extract.

Total phenolic compound content

Total phenolic content was determined using standard Folin–Ciocalteuprocedure (Ghasemi et al., 2009). Tea leaf extracts were prepared from the concentration of 1-100 mg/ml. The sample, 500 µl was added to the mixture of folin-ciocalteu, 50% (125 µl), deionized water (1,250 µl) and 95% ethanol (250 µl). The mixture was incubated in the dark for 5 minutes at room temperature. Then, Na_2CO_3, 5% (250 µl) was added to stop reactions by incubation in the dark for 30 minutes at room temperature. Absorbance was measured at 725 nm. Gallic acid was used as a standard compound. The content of total phenolic compound was determined and expressed as mg of gallic acid equivalent (GAE per 1 g of extract.

Total flavonoid compound content

Flavonoid compound was investigated using standard procedure (Ghasemi et al., 2009). Tea leaf extracts were prepared from the concentration of 1-100 mg/ml. The sample, 500 µl was added to 10% aluminium chloride (100 µl), methanol (1,500 µl),

1M potassium acetate (100 µl) and deionized water (2,800 µl). The mixture was incubated in the dark for 30 minutes at room temperature and absorbance was measured at 415 nm. Quercetin was used as a standard compound.

Results

Antibacterial susceptibility testing of tea leaf extracts by agar disc diffusion assay

The results of antibacterial susceptibility testing by agar disc diffusion were shown. Green, oolong, black tea leaves were extracted with water for 1 and 3 hours and the 500 mg/ml of aqueous extracts were tested against enteric pathogenic bacteria; *Escherichia coli*, *Salmonella typhimurium, Shigella dysenteriae, Staphylococcus aureus* and *Vibrio cholerea*.

The results showed that all bacteria were inhibited by green tea leaf extract after extraction for 1 hour with inhibition zones ranging from 7±6.1 mm. to 14±5.3 mm. *E. coli*, *S. aureus* and *V. cholerea* were inhibited by oolong tea leaf extract with inhibition zones ranging from 7±0.6 mm. to 11±0.1 mm whereas *E. coli*, *Sal. typhimurium* and *Shi. dysenteriae* were inhibited by black tea leaf extract with inhibition zones ranging from 7±4.9 mm. to 10.3±0.2 mm. Gentamycin (10 µg/ml) was used as positive control. (Table 1)

After extraction of tea leaves for 3 hours, the extracts were also tested against the pathogenic enteric bacteria. The results showed that all bacteria were inhibited by green tea and oolong tea leaf extracts with inhibition zones ranging from 7±6 mm. to 14±5.7 mm. However, all bacteria except *S. aureus* were inhibited by black tea leaf aqueous extract with inhibition zones ranging from 6.9±6.0 to 10.1±10 mm (Table 2).

Table 1 Antibacterial susceptibility testing by agar disc diffusion assay of tea leaf extracts after extraction for 1 hour

Bacteria	Zone of inhibition (mm)			Gentamycin (10 µg/ml)
	Green	Oolong	Black	
E. coli	7.27±5.1	7±0.6	7±4.9	20
S. aureus	10.8±0.1	11±0.1	0	23
Sal. typhimurium	7.0±6.1	0	7.7±0.5	20
Shi. dysenteriae	7.3±6.3	0	10.3±0.2	20
V. cholerea	14±5.3	8.3±1.5	0	23

Table 2 Antibacterial susceptibility testing by agar disc diffusion assay of tea leaf extracts after extraction for 3 hours

Bacteria	Zone of inhibition for organisms (mm)			Gentamycin (10 µg/ml)
	Green	Oolong	Black	
E. coli	13.4±12	11.3±10	6.9±6.0	20
S. aureus	14±5.7	13.7±5.5	0	23
Sal. typhimurium	10.5±0.3	7±6	10.1±10	20
Shi. dysenteriae	10.1±10	9.2±1.3	7.2±6.3	20
V. cholerea	10.6±0.2	7.3±06	8.6±1.2	23

DPPH radical scavenging activity

The results obtained from DPPH assay were presented in Figure 1. The scavenging effects of tea leaf extracts after extraction for 3 hours were greater than those of tea leaf extracts after extraction for 1 hour. The DPPH scavenging activities of green tea, oolong tea and black tea leaf extracts after extraction for 3 hours were 80.38, 21.45 and 27.46 mg GAE/g

extract, respectively (Figure 1). After extraction for 1 hour, the DPPH scavenging activities of green tea, oolong tea and black tea leaf extracts were 46.13, 9.04 and 5.27 mg GAE/g extract, respectively.

Total phenolic compound content

The total phenolic contents of tea leaf extracts were determined using the Folin–Ciocalteu method. The total phenolic contents of green tea, oolong tea and black tea leaves after extraction for 3 hours were 12.86, 9.53 and 7.23 mg GAE/g extract, respectively. After extraction for 1 hour, total phenolic contents of green tea, oolong tea and black tea extracts were 10.90, 7.91 and 5.64 mg GAE/g extract, respectively (Figure 2).

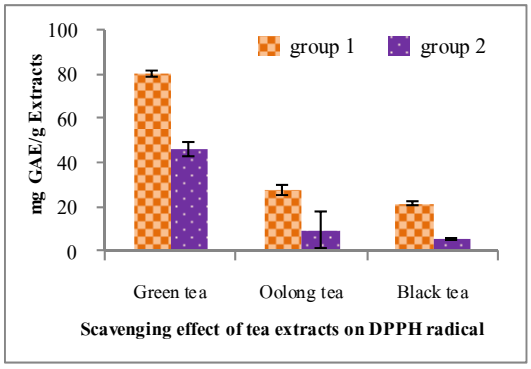

Figure 1 The anti-free radicals activity of aqueous extracts of green tea, oolong tea and black tea leaves by DPPH assay after extraction for 3 hours (group 1) and 1 hour (group 2). All values are means (±SD) of triplicate measurements.

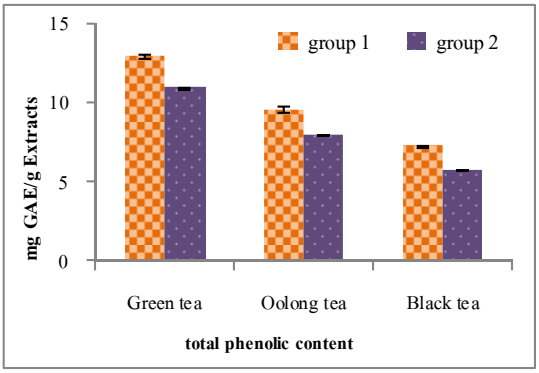

Figure 2 Total phenolic content of aqueous extracts of green tea, oolong tea and black tea leaves after extraction for 3 hours (group 1) and 1 hour (group 2). All values are means (±SD) of triplicate measurements.

Total flavonoid compound content

Total flavonoid contents of tea leaf extracts were shown in Figure 3. The total flavonoid contents of green tea, oolong tea and black tea leaves after extraction for 3 hours were 105.42, 69.61 and 53.66 mg quercetin/g extracts, respectively. After extraction for 1 hour, total flavonoid contents of green tea, oolong tea and black tea extracts were 6.78, 6.17 and 5.70 mg quercetin/g extracts, respectively.

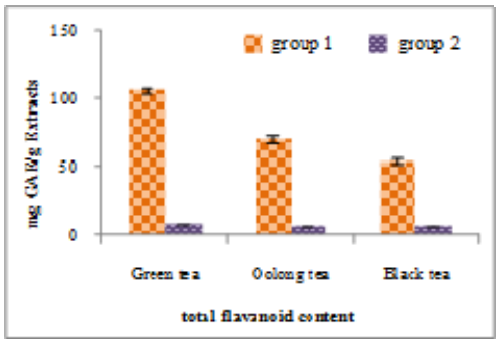

Figure 3 Total flavonoid content aqueous extracts of green tea, oolong tea and black tea leaves after extraction for 3 hour (group 1) and 1 hour (group 2). All values are means (±SD) of triplicate measurements.

Discussion and Conclusions

Green tea exhibited the highest antibacterial activity against pathogenic enteric bacteria tested in this study. Oolong tea and black tea showed lower activity. Polyphenols are the compound that mostly found in tea leaf. Several catechins include epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC) epicatechin-3-gallate (ECG), epicatechin (EC), and gallocatechin-3-gallate (GCG) (Kirk, Othmer, 1980). Catechins play an important role in inhibition of bacterial growth and damaged bacterial cytoplasmic membrane (Ikigai et al., 1993). Moreover, catechins can directly bind to bacterial

peptidoglycan and induce its precipitation. Previous study showed that major mechanisms of catechins for antibacterial activity against *Staphylococcus* was induction of the cell wall damage and interference with the cell wall biosynthesis through direct binding of catechins with peptidoglycan (Shimamura, Zhao, 2007). Moreover, another study suggested that the bactericidal action of EGCG might also depend upon the generation of hydrogen peroxide by the reaction of EGCG with reactive oxygen species in the presence of superoxide dismutase (Arakawa et al., 2004).

In our result, green tea extracts also showed the highest antioxidant activity. Total phenolic and flavonoid content of green tea leaf extracts were higher than oolong tea and black tea extracts. Green tea was found to be as a potent source of beneficial antioxidants, which also found in fruits and vegetables. Tea contained particularly rich in polyphenols, including catechins, theaflavins and thearubigins, which contributed to the health benefits of tea (Frei, Higdog, 2003).

Content of catechins in tea depends on types of the tea leaves (Lunder, 1992). Polyphenols were the most abundant group of compounds in tea leaf, and the catechins constituted the major component and seemed to be responsible for the antioxidant activity. There was a good correlation between the antioxidant activity and the epigallocatechingallate (EGCg) content. The content of catechins in tea was related to the degree of fermentation of tea during manufacture. Green tea was a non-fermented tea, thus it showed high content of catechins. Oolong tea was semi-fermented to permit a moderate level of enzymatic oxidation, while black tea was the most thoroughly oxidized enzymatically. The compound derived from fermentation of tea provided unique properties, which were different from non-fermented tea (Arakawa et al., 2004). Therefore, catechins, which contained antioxidant activity, were reduced after fermentation of tea leaves.

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