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Biochemical and antibacterial properties of Thai medicine herbal infusions

[1]Chunthanom, P., [2*]Chaikham, P. and [3]Intaket, R.

[1]Department of Food Science and Technology, Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon campus, Sakon Nakhon 47160, Thailand
[2]Science and Technology Research Institute, Chiang Mai University, Chiang Mai 50200, Thailand
[3]Department of Thai Traditional Medicine, Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon campus, Sakon Nakhon 47160, Thailand

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Abstract

Biochemical and antibacterial properties of Thai medicine herbal extracts including chrysanthemum, lotus stamen, bullet wood, cananga, safflower, roselle and licorice as well as cashew flower, leaf buds and leave were investigated. It was found that dried cashew flower, leaf buds and leave extracts contained highest amount of total phenolics, tannin and 2,2-diphenyl-1-picrylthydrazyl (DPPH) radical inhibition compared to the other herbs. *Escherichia coli* inhibition of cashew flower, cashew leaf buds and cashew leave extracts was higher than others. The flavored herbal infusion, mixture of cashew flower, licorice root, cashew leaf buds and cashew leave (7:1.25:1.25:0.5 by weight), had high quantities of total phenolics, tannin and antioxidant capacity (DPPH assay) as well as antibacterial activity than those of Thai green tea extract. In overall the highest antimicrobial activity occurred in flavored herbal infusion was related with the highest tannin content and acidity.

Introduction

Up to date, the search for new natural antioxidant and antimicrobial agents from plants has been interested. In a search for new plant products derived photochemical or biological active compounds against chronic and degenerative diseases (Fu *et al*., 2011). Antioxidant compounds, potential agents are the most important in plants due to their ability to reduce free radical mediated degradation of cells and tissues in an organism (Almajano *et al.*, 2008). The antioxidant action mechanism is to scavenging free radicals, inhibiting oxidation and antimicrobial activity in cells (Frei and Higdon, 2003) and also treating oxidative stress related diseases (Fu *et al.*, 2011).

Antioxidant capacity of the dietary herbal plant is the most important due to phenolic compounds including flavonoids, phenolic acids, tannins and phenolic diterpenes (Polterait, 1997). Tannin is defined as water-soluble phenolic compound, to serve as a biological antioxidant (Haslam, 1996). Further studies have revealed that phenolics have antimicrobial activities (Almajano *et al*., 2008). It is necessary to maintain microbiological safety and minimize the food-borne microorganisms (Almajano *et al*., 2008). Many studies have investigated the effect of polyphenols on intestinal pathogens. The bacteria tolerance to polyphenols depends on the bacterial species and the structure of polyphenol (Campos *et al.*, 2003; Taguri *et al*., 2004). Herbal teas are good sources of antioxidants and antimicrobial with positive effects on human health for preventing and treating many diseases (Almajano *et al.*, 2008). Several studies show the tea extracts act as food pathogens inhibitors, including *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Salmonella* sp., *Staphylococcus aureus*, *Shigella disenteriae*, *Vibrio cholera*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Clostridia* and *Helicobacter pylori* (Nagi *et al.*, 2003; Taguri *et al*., 2004; Gramza and Korczak, 2005). On the other hand, several researchers have found that green tea extract was not effective against *E. coli* (Toda *et al*., 1989; Nazer *et al*., 2005).

Various kinds of herbs have been used to make infusions. Medicine herbal plants including chrysanthemum, lotus stamen, bullet wood, cananga, safflower, roselle, licorice and cashew are also drink in Thailand. Chrysanthemum (*Chrysanthemum indicum* L.) is widely used in Chinese traditional medicine for the treatment of inflammation, hypertension and respiratory diseases (Cheng *et al*., 2005). It was also found to have antimicrobial and antioxidant action

*Corresponding author.
Email: *osirismu@hotmail.com*

(Cheon *et al.*, 2009; Pongjit *et al.*, 2011). Lotus stamen (*Nelumbo nucifera* Gaertn.) is particularly valued for Thai medicinal properties. Their interesting bioactivities such as antihypertensive, hypertropic scar fibroblast inhibiting, antidepressive, serotonin antagonist, anticancer, antispasmodic, antioxidativeand antimicrobial activities were mentioned (Martin *et al*., 1993; Kim *et al.*, 1998; Ohsugi *et al*., 1999; Burns et al., 2000; Ahmad and Beg, 2001; Jung *et al.*, 2003). Other Thai herbs, such as bullet wood (*Mimusops elengi* L.), cananga (*Cananga odorata*), safflower (*Carthamus tinctorius* L.), roselle (*Hibiscus subdariffa* L.), licorice (*Glycyrrhiza glabra* Linn.) and cashew (*Anacardium occidentale* L.), also contain variety of bioactive components and thus possess various kinds of biological and pharmacological activities including antibacterial, antihemorrhoidal, antifungal, anticariogenic, free radical scavenging, antihyperglycemic, antineoplastic and antiviral activities as well as gastroprotective property (Wang *et al.*, 2001; Friis-Møller *et al.*, 2002; Sacchetti *et al.*, 2005; Yen *et al*., 2005; Trevisan *et al*., 2006; Bozan and Temelli, 2008; Chaovanamethakul *et al*., 2008; Manjeshwar *et al.*, 2011; Gami *et al.*, 2012; Liu *et al*., 2012).

There is lack of data information on synergy between plant herbal extracts and antibiotic drugs. The objective of this work was to evaluate the biochemical properties and antibacterial activity of some Thai herbal plants for investigation the relationship between biochemical property and antibacterial effect. We have carried out a study on some herbal plants to evaluate an appropriate for the flavored herbal plant comparison with Thai green tea.

Materials and Methods

Herbal preparation and extraction

The herbal plants including chrysanthemum, lotus stamen, bullet wood, cananga, safflower, roselle flower, licorice root and green tea were purchased from local market in Sakon Nakhon, Thailand. To prepare freeze dried cashew flower, the fresh cashew flower was harvested, soaked in tap water and then lyophilized using a freeze dryer (alpha 1-2 LD plus, Germany) at -50°C for 12 h or overnight. For conventional drying, the flower was dehydrated using a tray dryer (Mammert, Germany) at 50°C for 36 h. Besides cashew flower, its leaf buds and leave were dried using the tray dryer at the same condition. The herbal plant was powdered using a National blender model MX-20G (250 W, National, Thailand) at low speed for 2 min.

To extract bioactive components, 2 g of blended sample were added into 100 mL hot water (98-100°C) and soaked for 5 min. The extracts were filtered through Whatman No.2 filter paper (Whatman, Spain).

Determination of total phenolics

Total phenolic contents were determined using Folin-Ciocalteu assay as described by Singleton and Rossi (1965) with some modifications. An aliquot of 25 µL extract was added into 9.975 mL deionized water (RCI Lab-Scan, Thailand) and mixed with 0.25 mL of Folin-Ciocalteu phenol reagent (Merck, Germany). The mixture was allowed to react for 5 min and then 1 mL of 10% Na_2CO_3 solution (Univar, New Zealand) was added. Consequently, the mixture was adjusted the total volume to 25 mL with deionized water and incubated in dark for 1.5 h at room temperature. The apparent blue complex was determined at λ_{max} 760 nm (Spectrophotometer, Perkin Elmer Lamda 25, USA). Total phenolics were expressed as mg gallic acid equivalent per gram of dry sample (mg GAE/g).

Determination of tannin content

Tannin content was determined following the modified method described by Schanderl (1970). Two hundred microliters of the extract were added into 2.5 mL Folin-Denis reagent, mixed well for 3 min and 5 mL of sodium carbonate (Merck, Germany) solution were then admixed. The mixture solution was made up to 50 mL with distilled water and incubated at room temperature for 30 min. Absorbance of the solution was measured at λ_{max} 760 nm (Spectrophotometer, Perkin Elmer Lamda 25, USA). Tannin content was expressed in mg of tannic acid equivalents per gram dry sample (mg TAE/g).

Determination of antioxidant capacity

2,2-Diphenyl-1-picrylthydrazyl (DPPH) radical scavenging activity was determined following the methods described by Singh *et al.* (2002) with some modifications. One hundred microliters of the sample extract were added into 5.9 mL of 40% ethanol (Chemical & Lab Supplies, Thailand) and continuously stirred for 5 min. Subsequently, the mixture was mixed with 100 µL of 1.5 µM DPPH radical (Sigma, Germany) in 95% ethanol (CARLO ERBA, France), shaken vigorously and allowed to stand for 30 min at room temperature. Absorbance of the solution was measured at λ_{max} 517 nm (Spectrophotometer, Perkin Elmer UV WINLAB, USA). A control was prepared using 0.1 mL ethanol. Percentage inhibition of DPPH radicals was calculated using the following formula:

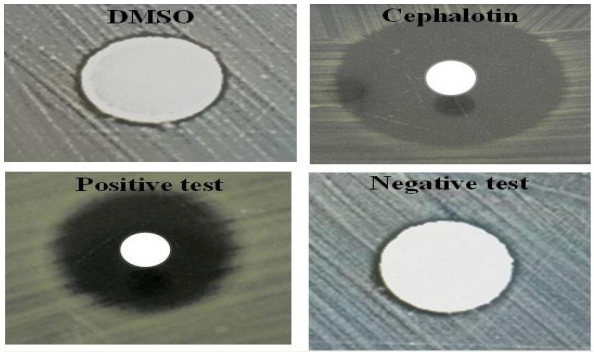

Figure 1. Agar diffusion test shown clear zone of inhibition surrounding the herbal extract discs

DPPH radical scavenging activity (%) = [1 - (Abs_{sample}/$Abs_{control}$)] x 100, where Abs is the absorbance.

Antimicrobial activity assessment

The microorganism used in this study was *E. coli* TISTR 780. This microbe was obtained from the Thailand Institute of Scientific and Technological Research (TISTR, Thailand). Ten grams of each herbal powder were soaked into 500 mL of boiling water for 5 min and then filtered through Whatman No.2 filter paper. The filtrate was dehydrated using a rotary evaporator (Eyela OSB-2100 CCA-1111, USA) at 50°C until dry. The dried extract was dissolved in dimethyl sulfoxide (DMSO) (Fisher Scientific, UK) at the concentration of 30, 300 and 3,000 µg/mL, subsequently sonicated at 40°C for 15 min.

Antagonistic activity was determined following the agar diffusion method as described by Pranoto *et al.* (2005) with some modifications. *E. coli* was grown at 35°C for 24 h in nutrient broth and agar (HiMedia, India). The density of microbial required for the test was adjusted to 0.5 McFarland standards, which corresponded to approximately 1.0 x 10^8 CFU/mL. This estimation required the researcher to visually assess the turbidity level in nutrient broth to be the equal to that of the standard. The culture in broth was spread on agar sterile plastic Petri plates using the sterile cotton bud. The sterile antimicrobial susceptibility test discs (6 mm diameter, Whatman, England) were placed on the spread-culture agar and the various extract concentrations (~ 20 µL) were then pipetted to each disc. Cephalotin (antibiotic drug, Oxoid, UK) and DMSO solution were used as a positive and negative control, respectively. All plates were incubated at 35°C for 48 h under aerobic environment. Clear zones surrounded positive herbal extract discs were appeared after the incubation period (Figure 1). The inhibition zone ratio was calculated using the following formula:

Inhibition zone ratio = [diameter of inhibition zone of sample (mm)]/[diameter of inhibition zone of antibiotic drug (mm)].

Table 1. Biochemical and antioxidant properties of various Thai herbal extracts

Infusions	Total acidity (%)	pH	Total phenolics (mg GAE/g)	Tannin (mg TAE/g)	DPPH inhibition (%)
Chrysanthemum	0.03 ± 0.01^{c}	5.62 ± 0.08^{a}	0.21 ± 0.02^{de}	41.70 ± 0.88^{e}	22.73 ± 1.91^{e}
Lotus stamen	0.11 ± 0.04^{ab}	5.61 ± 0.10^{a}	0.26 ± 0.10^{d}	53.39 ± 2.70^{d}	65.52 ± 1.75^{b}
Bullet wood	0.11 ± 0.04^{ab}	5.06 ± 0.06^{c}	0.12 ± 0.08^{e}	38.93 ± 0.81^{e}	6.74 ± 1.19^{f}
Cananga	0.06 ± 0.01^{b}	5.23 ± 0.05^{b}	0.25 ± 0.01^{d}	39.21 ± 1.27^{e}	28.04 ± 1.43^{e}
Safflower	0.15 ± 0.04^{ab}	5.04 ± 0.10^{c}	0.83 ± 0.03^{b}	56.01 ± 0.76^{d}	60.87 ± 0.77^{c}
Roselle	0.17 ± 0.04^{a}	2.78 ± 0.06^{f}	0.38 ± 0.02^{c}	40.52 ± 1.47^{e}	59.44 ± 1.60^{c}
Licorice root	0.18 ± 0.03^{a}	4.97 ± 0.12^{c}	1.35 ± 0.01^{a}	12.52 ± 0.43^{f}	41.35 ± 2.37^{d}
Tray-dried cashew flower	0.13 ± 0.06^{ab}	4.03 ± 0.08^{d}	1.31 ± 0.08^{a}	137.06 ± 1.98^{a}	93.20 ± 0.52^{a}
Freeze-dried cashew flower	0.11 ± 0.04^{ab}	4.02 ± 0.02^{d}	0.88 ± 0.04^{b}	137.20 ± 0.95^{a}	92.18 ± 0.54^{a}
Cashew leaf buds	0.15 ± 0.05^{ab}	3.94 ± 0.04^{d}	1.48 ± 0.01^{a}	103.33 ± 8.24^{b}	94.64 ± 0.57^{a}
Cashew leave	0.17 ± 0.08^{a}	3.77 ± 0.04^{e}	1.44 ± 0.01^{a}	85.67 ± 2.67^{c}	95.92 ± 0.53^{a}

Means in the same column followed the same letter indicating an insignificant difference (P > 0.05). Each data point is the average of three replications.

Statistic analysis

All data were the means of triplicate determinations with standard deviations (means ± SD). Analysis of variance (ANOVA) was carried out using ANOVA by Minitab release 14, and determination of significant differences among treatment means was done by Tukey's test ($P < 0.05$). The relationship between biochemical properties and antibacterial effects was evaluated using linear regression analysis.

Results and Discussion

Biochemical and antioxidant properties of herbal extracts

Table 1 exhibits acidity content of licorice root, roselle and cashew leave extracts was significantly higher ($P < 0.05$) than cananga and chrysanthemum, which roselle extract showed lowest in pH. Dried cashew flower, leaf buds and leave extracts has high levels of total phenolics, tannin content and DPPH radical inhibition. For dried cashew flower, total phenolics in tray-dried cashew flower were comparable to those observed in licorice root, cashew leaf buds and cashew leave extracts, whereas these compounds in chrysanthemum and bullet wood extracts were significantly lower ($P < 0.05$). Tannin content in both dried cashew flower extracts was significantly higher ($P < 0.05$) than others. Antioxidant capacity as DPPH radical inhibition was highest in cashew flowers, leaf buds and leave infusion. It was interesting to note that biochemical and antioxidant properties depended on the kind of herbal plants.

In general, cashew flower treated with long thermal exposure in tray dryer did not affect the tannin content and DPPH radical inhibition while it was increase in total phenolics content compared with freeze dryer. Ahmad-Qasem *et al.* (2013) dried olive leaves using freeze drying and hot air drying, and found that hot air dried sample at 120°C showed the highest total phenolic compounds. Hossain *et al*. (2010) stated that both drying methods make

Table 2. Inhibition zone ratio of Thai herbal extracts against *E. coli* TISTR 780

Plant extracts	Plant extracts concentration (µg/mL)		
	30	300	3,000
	E. coli	*E. coli*	*E. coli*
Chrysanthemum	-*	-	0.34 ± 0.04^{ab}
Lotus stamen	-	-	0.13 ± 0.22^{b}
Bullet wood	-	-	-
Cananga	-	-	0.13 ± 0.23^{b}
Safflower	-	-	0.23 ± 0.21^{b}
Roselle	0.35 ± 0.05^{b}	0.38 ± 0.03^{b}	0.55 ± 0.14^{a}
Licorice root	-	-	-
Tray-dried cashew flower	0.37 ± 0.02^{b}	0.43 ± 0.02^{b}	0.50 ± 0.01^{a}
Freeze-dried cashew flower	0.50 ± 0.01^{a}	0.52 ± 0.06^{a}	0.57 ± 0.05^{a}
Cashew leaf buds	-	0.44 ± 0.10^{b}	0.60 ± 0.08^{a}
Cashew leave	-	0.46 ± 0.12^{ab}	0.80 ± 0.35^{a}

Means in the same column followed the same letter indicating an insignificant difference (P > 0.05). Each data point is the average of three replications. * - = no inhibition zone.

Table 3. Biochemical and antioxidant properties of Thai green tea and flavored herbal infusion

Plant extracts	%Acidity	pH	Total phenolics (mg GAE/g dw)	Tannin (mg TAE/g dw)	%DPPH inhibition
Thai green tea	0.05 ± 0.01^{b}	5.81 ± 0.01^{b}	0.76 ± 0.26^{b}	50.17 ± 2.93^{b}	25.32 ± 0.12^{a}
Flavored herb	0.14 ± 0.10^{a}	3.61 ± 0.03^{b}	1.57 ± 0.12^{a}	185.31 ± 6.89^{a}	97.11 ± 0.44^{a}

Means in the same column followed the same letter indicating an insignificant difference (P > 0.05). Each data point is the average of three replications.

Table 4. Clear zone ratio of Thai green tea and flavored herbal infusion against *E. coli* TISTR 780

Plant extracts	Plant extracts concentration (µg/mL)		
	30	300	3000
Thai green tea	0.10 ± 0.17^{b}	0.23 ± 0.11^{b}	0.44 ± 0.03^{b}
Flavored herb	0.46 ± 0.02^{a}	0.45 ± 0.05^{a}	0.55 ± 0.06^{a}

Means in the same column followed the same letter indicating an insignificant difference (P > 0.05). Each data point is the average of three replications.

the release of phenolic compounds into the solvent easier due to the cell wall breakdown related to water removal. The drying not only facilitates the extraction of phenolic compounds, but also the release of oxidative enzymes such as polyphenoloxidase and peroxidase, which would reduce these components during the extraction process. While the high temperatures involved during hot air drying could deactivate the enzymes, thus avoiding the phenol degradation (Ahmad-Qasem *et al*., 2013). In general, freeze-drying is a better drying method compared with thermal drying (Chong and Lim, 2012). Several researchers reported that the antioxidant activity and total phenolics of freeze dried samples higher than tray drying (Bodo *et al*., 2004; Chang *et al.*, 2006; Chan *et al.*, 2009).

Health-promoting properties of these herbal extracts were studied. Licorice root and cashew extracts demonstrate antiinflammation, anti-ulcer, antiviral, anti-atherogenic, anticarcinogenic, antimicrobial and antioxidant activities (Demizu *et al*., 1988; Vaya *et al*., 1997; Belinky *et al*., 1998; Wang *et al.*, 2001; Friis-Møller *et al*., 2002; Trevisan *et al*., 2006). While, roselle can prevent cancer and lower blood pressure as well as improve the digestive system in human (Mohammed *et al.*, 2007). Bullet wood and other herbs contain high phenolics, which can serve as a good natural source of antioxidants in the foods and pharmaceutical industries (Sacchetti *et al.*, 2005; Yen *et al*., 2005; Bozan and Temelli, 2008; Chaovanamethakul *et al.*, 2008; Liu *et al.*, 2012).

Antibacterial activity of herbal extracts

Antibacterial effect of various herbal extracts is shown in Table 2. Comparison of plant extracts, various concentrations of both dried cashew flower extracts were higher inhibition, showing the largest zone. It was worth noting that freeze-dried cashew flower extract trended to greater inhibition effect than tray-dried cashew flower extract. However, at the highest concentration the *E. coli* inhibition was significantly higher ($P < 0.05$) in cashew leaf buds, cashew leave, both dried cashew flower and roselle extracts than the other herbs. At low and mild concentrations (30 and 300 µg/mL) chrysanthemum, lotus stamen, bullet wood, licorice root and cananga, the extracts did not have an inhibitory effect against *E. coli*, while the inhibition effect of cashew flower extracts at all concentrations were observed, in particular freeze-dried cashew flower extract. In overall, inhibition effect of the herbal extracts depended on the kinds and the concentrations. For cashew flower extract, the decrease of microbial inhibition efficiency might be due to the thermal degradation of some bioactive compounds during tray drying. Besides Thai herbal plants, Romero *et al.* (2005) found that herbal remedies used in South Texas were inhibited various pathogenic bacteria including *S. aureus*, *Pseudomonas aeruginosa* and *E. coli*. Similar results were reported by Shikanga *et al.* (2010) with South African Lippia herbal infusions. In addition, Li *et al.* (2005) reported that aqueous extracts of some Chinese herbal medicines including *Cassia obtusifolia* (Fabaceae), *Fritillaria thunbergii* and *Eugenia caryophyllata* were remarkably inhibitory against *H. pylori* strains.

In this study, the correlation of some biochemical properties and antimicrobial activity of herbal extracts was observed. However, this experiment was found that no correlation between *E. coli* inhibition and antioxidant activity, but correlated with some chemical properties including acidity and pH, in particular roselle and licorice root extracts. The *E. coli* inhibition corresponded to the highest acidity (low pH). No correlation between microbial inhibition and total phenolics or tannin content or antioxidant capacity was seen. This might be due to the influence

of other compounds that have an inhibitory effect (Boulekbache-Makhlouf *et al.*, 2013).

In all over, tray-dried cashew flower, cashew leaf buds and cashew leave extracts were the best microbiological inhibitor and high concentration of total phenolics, tannin content and DPPH radical inhibition. The microbiological inhibition of freeze-dried cashew flower was higher than tray-dried cashew flower, while total phenolics in tray-dried flower were higher than those of the freeze-dried flower. Some active compound of freeze-dried sample may act as antimicrobial agent. Dixon and Paiva (1995) reported that flavonols including quercetin and kaempferol are antimicrobial compounds, they are unstable in heat treatment. Tray drying may cause losses in some antimicrobial agent. However, the production cost of tray drying was lower than freeze drying. Thus, tray-dried cashew flower was suitable for mixing with other ingredients including licorice root, cashew leaf buds and leave at a ratio of 70:12.5:12.5:5 by weight respectively, for flavored herbal processing which the most panelist acceptance (previous study).

Properties of flavored herbal infusion

The results in Table 3 showed flavored herbal infusion had high levels of total phenolics, tannin content and DPPH radical inhibition than those in Thai green tea extract, while pH was lowest ($P < 0.05$). Besides biochemical properties, flavored herbal extract at high concentrations (300-3000 µg/mL) was inhibited the microbe better than green tea ($P < 0.05$) (Table 4). In general green tea extract at all concentrations did have an inhibitory effect against *E. coli*. Almajano *et al.* (2008) reported that white tea and green tea extracts were inhibited the growth of *E. coli*. The *E. coli* inhibition of flavored herbal infusion and green tea infusion apparently rose with an increasing of the extract concentration. In overall flavored herbal infusion had high quantifies of total phenolics, tannins and DPPH radical inhibition as well as greater antibacterial effect than those of Thai green tea extract. The highest antimicrobial activity occurred in flavored herbal infusion was related with the highest tannin content and acidity.

Conclusion

Dried cashew flower, leaf buds and leave extracts showed the highest amount of total phenolics, tannin and DPPH radical inhibition compared to the other herbs. *E. coli* inhibition of cashew flower, cashew leaf buds and cashew leave extracts was significantly higher than others. The flavored herbal infusion, mixture of cashew flower, licorice root, cashew leaf buds and cashew leave (7:1.25:1.25:0.5 by weight), had high quantities of total phenolics, tannin and DPPH radical inhibition as well as antibacterial activity than those of Thai green tea extract. The highest antimicrobial activity occurred in flavored herbal infusion was related with the highest tannin content and acidity. Flavored cashew tea is a new product in the marketplace, which contributes to an increase of an essentially economical value of cashew products. Thus, the consumer acceptability of this product should be further evaluated.

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