Antibacterial activity of Local Herb Extracts against *Escherichia coli* ATCC25822, *Salmonella sp.*, *Bacillus cereus*, and *Listeria monocytogenes* 10403S

By

Ms. Sasiwan Piya-isarakul

ID.521-8282

A special project submitted to the Faculty of Biotechnology, Assumption University in part of fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology

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By	:	Ms. Sasiwan Piya-isarakul
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Level of study	:	Bachelor of Science
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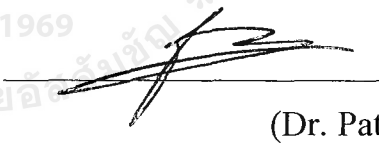

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ABSTRACT

In Thailand, herbs are used as traditional medicine since the ancient times therefore primary health care for local people. The leaves, roots, bark, seeds, and whole part are source of herbal medicines. The objectives of this study are to study the individual antibacterial activity of seven Thai local herbs against foodborne pathogenic bacteria and to study the effect of five extraction conditions on antibacterial activity of each herb. The seven herbs; *Tradescantia spathacea* (Oyster plant), *Andrographis paniculata* (Kariyat), *Clinacanthus nutans* (Sabah snake grass), *Eleocharis acicularis* (Needle- Spike Rush), *Acacia concinna* (Soap pod), *Phyllanthus niruri* (Stonebreaker), and *Tinospora cordifolia* (Gulancha) were extracted under five extraction conditions; 95% ethanol, chloroform, sterile distilled water, autoclaving at 121°C 15 PSI for 15 minutes, and hexane. All herbs were in vitro screened for antibacterial activity against six bacteria; *Escherichia coli* ATCC25822, *Samonella enterica* Typhimurium U302 (DT1046), *Samonella enterica* Enteritidis (human), *Samonella enterica* 4,5,12: i- (human) US clone, *Bacillus cereus*, and *Listeria monocytogenes* 10403S by using agar disc diffusion method. The result of in vitro antibacterial screening showed 95% Ethanol and Chloroform extraction condition give the highest antibacterial activity of all herbs against all bacteria in this study. The range of antibacterial activity is between 8.5 mm to 11.5 mm. The highest antibacterial activity was of *C. nutans* chloroform crude extracted against *S. enterica* Typhimurium U302 (DT1046); 11.50±1.29 mm. The range of MIC and MBC is between 32 µl/ml to 256 µl/ml. The active compounds of each herbs will be further investigated by GS-MS. These results showed the promising of antibacterial activity of all Thai local herbs which are stepping stone for further application like food industry, pharmaceutical industry, and cosmetic industry.

Keywords: Antibacterial activity, Thai local herb, Pathogenic bacteria

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INTRODUCTION

In modern society, food safety is highly concerned that focused on the pathogenic bacteria. The pathogenic bacteria are caused foodborne illness and many outbreaks that have been spread in our world.

Escherichia coli is Gram-negative, rod-shaped bacteria, and harmless. It is well known that some species of *E. coli* produce shiga toxin and the gastrointestinal system flora of some domestic animals and human. Despite 150 such *E. coli* serotypes that cause disease in humans. The contaminated food and water are the source of *E. coli*, such as undercooked ground beef, raw milk and juice, untreated water and swimming in contaminated water. *E. coli* infection can cause for feces of people [1],[41].

Salmonella is Gram-negative, rod-shaped, and non-sporeforming bacterium. This bacterium is in the family of *Enterobacteriaceae* and the genus *Salmonella* is divided into two species which are *S. enteric* and *S. bongori* that can cause illness in humans. *S.* Enteritidis *and S.* Typhimurium is the one of subspecies *Salmonella* that referred to their surface and flagella antigenic properties. *Salmonella* causes two kinds of illness: Gastrointestinal (nausea, vomiting, diarrhea, and fever) and Typhoidal illness (high fever, headache, and lethargy). In people who are weak in immune systems, *Salmonella* can spread to other organs and cause very serious illness [1].

Bacillus cereus is a Gram-positive, rod-shaped, facultative anaerobic, and endosporeforming bacterium. *B. cereus* is widespread in the environment and often is isolated from soil and vegetation. There are two types of food-borne illnesses; Emetic form (short-incubation): nausea and vomiting and abdominal cramps, and Diarrheal form (long-incubation): abdominal cramps and diarrhea [1].

Listeria monocytogenes is a Gram-positive, rod-shaped, and facultative bacterium; it is salt-tolerant and not only can survive in temperatures below 1°C. *L. monocytogenes* can causes of death from foodborne illness. There are two forms of disease; it can range from mild to intense symptoms of nausea, vomiting, aches, fever, and diarrhea. The other, more deadly form occurs with the infection through the bloodstream to the nervous system including the brain that is resulting in the meningitis [1].

Herb is one type that becomes the natural source of antibiotics and medicinal properties. It have active compound that effective to antimicrobial. The seven herbs; *Tradescantia spathacea* (Oyster plant), *Andrographis paniculata* (Kariyat), *Clinacanthus nutans* (Sabah snake grass), *Eleocharis acicularis* (Needle- Spike Rush), *Acacia concinna* (Soap pod), *Phyllanthus niruri* (Stonebreaker), and *Tinospora cordifolia* (Gulancha) were used in this study. This research was study the individual antibacterial activity of three Thai local herbs against six foodborne pathogenic bacteria under five extraction conditions.

OBJECTIVES

- To study the individual antibacterial activity of seven local herbs; *Tradescantia spathacea*, *Andrographis paniculata*, *Clinacanthus nutans*, *Eleocharis acicularis*, *Acacia concinna*, *Phyllanthus niruri*, and *Tinospora cordifolia* against foodborne pathogenic bacteria.

- To study the effect of extraction conditions; 95% Ethanol, Sterile Distilled water, Autoclaving at 121°C for 15 minutes, Chloroform and Hexane on antibacterial activity of each herbs against foodborne pathogenic bacteria.

LITERATURE REVIEW

Escherichia coli

The symptoms of *E. coli* infectious severe diarrhea which may range from mild, watery to bloody, abdominal pain, nausea and vomiting, also presenting of little or no fever [3]. The symptoms of HUS included decreased urine production, dark or tea-colored urine, and facial pallor [2]. The symptoms of *E. coli* usually start from 3 or 4 day and the symptoms are recovered within 5 to 10 days. The ability of exposure included contaminated food or water, and spread has occurred [2].

Outbreak of *Salmonella* sp.

Salmonella is an invasive bacterium and it causes human infection as known as salmonellosis. The symptoms of *Salmonella* included diarrhea, abdominal pain, headache, and prolonged high fever. The incubation period from 1 to 7 weeks and the illness usually lasts from 1 to 8 weeks [11]. The official reports of *Salmonella* outbreak are much available.

- *Salmonella* Typhimurium Infections Linked to Peanut Butter (2009)
  September 1, 2008 to March 31, 2009. Patients range in age from under or 1 to 98 years; 21% are age under 5 years, 17% are upper 59 years, and 48% of patients are female. The 24% of patients were hospitalized and caused nine deaths [8].

- *Salmonella* Enteritidis Infections Linked to Alfalfa Sprouts and Spicy Sprouts (2011)
  The illnesses occurred between April 12 and June 15, 2011, the patients range in age from 12 years to 77 years old, 3 patients were hospitalized and no death [9].

- *Salmonella* Typhimurium Infections Linked to Live Poultry in Backyard Flocks (2013)
  This outbreak had 316 persons infected with the outbreak strain of *Salmonella* Typhimurium, 51 patients were hospitalized and no death; 81% of patients had contact with live poultry in the week before their illness began and 97% of patients had purchasing live poultry from agricultural feed stores [12].

Outbreak of *Bacillus cereus*

The symptoms of *B. cereus* included watery diarrhea, abdominal cramps, emetic, nausea, and vomiting. The incubation period before onset of disease is 8 to 16 hours and the illness usually lasts for 12 to 14 hours. Spores are able to survive harsh environments including normal cooking temperatures. The official reports of *B. cereus* outbreak are much available. In August 2003, five children of a family became sick after eating pasta salad. A fatal case due to liver failure after the consumption of pasta salad is described and demonstrates the possible severity of the emetic syndrome. Moreover, young girl age is 7 years old; she died at 13 hours after the meal [16].

Outbreak of *Listeria monocytogenes*

The infection of *L. monocytogenes* is invasive infection, the resulting disease as known as listeriosis. The incubation period of literiosis is one to several weeks. The most susceptible to the disease are children under 4 years old, pregnant woman, and the elderly [15]. The official reports of *L. monocytogenes* outbreak are much available.

- Listeriosis Linked to Imported Frescolina Marte Brand Ricotta Salata Cheese (2012)
  The 22 persons are infected with the outbreak-associated strain of *Listeria monocytogenes*; 20 patients were hospitalized and 4 patients were death (two of these deaths were listeriosis) [13].

- *Listeria Monocytogenes* Linked to Cheese (2013)
  July 3 2013, Five patients were hospitalized, and one death. One patient in a pregnant women resulted in a miscarriage [14].

Tradescantia spathacea

T. spathacea Kerr. is in the Family Commelinaceae. In Thai medicine properties, it is used to relieve fever, coughing phlegm with blood, bronchitis cough, bacillary dysentery, and blood in the stool [37]. It also reported to possess of antimicrobial, insecticidal, anti inflammatory, anticancer and anti-fertility activities [37]. In the experiment, this herb was investigated to screen and elucidate in vitro effects of the above Thai medicinal plants on human lymphocyte

proliferation and functions of natural killer (NK) cells [37]. *T. spathacea* Kerr. was collected from Nonthaburi provience, Thailand which is used leaf part and water as solvent. Lymphocyte proliferative response to the extract was performed as described with the extracts at final concentrations of 1 ng/ml, 10 ng/ml, 100 ng/ml, 1 μg/ml, 5 μg/ml, 10 μg/ml and 100 μg/ml in complete RPMI 1640 containing 10% FBS [37]. As the result, demonstrate various patterns in stimulating effects of *T. spathacea* on the proliferative responses of human lymphocytes. *T. spathacea* significantly enhanced lymphocyte proliferation at the concentrations of 1 ng/ml, 10 ng/,l and 5 μg/ml, 10 μg/ml, and100 μg/ml[37].

Andrographis paniculata

In traditional systems of medicine, *A. paniculata* has been used to treat various conditions of infectious. In the modern research has investigated this herb for activity against various bacteria, parasites, and viruses. For the crude powder suspended in water, it was reported to *in vitro* antibacterial activity at concentration of 2 mg/mL crude powder against on *Salmonella, Shigella, E. coli*, gram A Streptococci, and *Staphylococcus aureus* [43]. The researcher had concluded that crude aqueous extract of leaves exhibit significant antimicrobial activity had no activity against on *E. coli* or *Klebsiella pneumonia* [20]. In contrast, it had against on gram positive *S. aureus*, methicillin-resistant *S. aureus* (MRSA), and gram-negative *Pseudomonas aeruginosa* [20]. The other study report that the ethanol extract of leaves have antibacterial activity against *S. aureus* and *E. coli* [22]. The study of ethanol extract of *A. paniculata* powder extracted is effective against *B. cereus* and *L. monocytogenes* [26].

Clinacanthus nutans

Thai medicinal plant is used for protection and treatment of viral diseases. *C. nutans* (Burm. f.) Lindau It has long been used in Thailand as a traditional medicine for the treatment of skin rashes, insect and snakebite, herpes simplex virus (HSV), and varicella-zoster virus (VZV) lesions [38]. So, in this experiment were study effects of compounds from *C. nutans* on DV2 infection in A549 cell line and determine mechanisms of the compounds from *C. nutans* on inhibition step of DV2 infection [38]. As the result, compounds 1-4 that were isolated from *C. nutans* leaves, their chemical compounds were investigated for anti-DV2 activity in A459 infected cell and the CC50 of the compound 1, 2, 3 and 4 showed 43, 25, 50, 50 μg/ml,

respectively. The sub-toxic concentration that used for test the anti-viral activity were 34, 5, 20, 25 μg/ml, respectively. It means that four compounds isolated from leave of *C. nutans* were evaluated for anti-DV2 activity [38].

Eleocharis acicularis

E. acicularis is found naturally growing shallow waterways. Aquascapers were inspired by the wispy, soft, and natural feeling so that *E. acicularis* brings to planted aquarium. *E. acicularis* looks similar to the other hairgrass species such as *E. parvula.* However, *E. acicularis* grows twice as taller which hight is 15 cm than *E. parvula* which is 6 cm [39]. In tropical country, it naturally growing in the field after harvest. The local people in locality at Pakthongchai prefecture, Nakhonratchasima provience, Thailand had inform the medicine property for this plant that is used for relieve fever, analgesic, and diuretic.

Acacia concinna

A. concinna is a tree native in Asian country. Its parts used as bark, leaves and pods. An infusion of the leaves is used in malarial fever [29]. A decoction of the pods relieves biliousness and acts as a purgative [29]. The previous study used dried powder of *A. concinna* under three extraction processes (Ethanol, Methanol and Chloroform) by using agar diffusion method. The result showed that antibacterial activity of all the extract showed good inhibitory activity against all the tested pathogens and the chloroform exhibited maximum antimicrobial activity comparative by better activity than the other extracts against *P.aerogenosa* and *S. aureus.* The activities of the extract were compared with standard antibiotics and results indicate that *A. concinna* Bark possesses potential broad spectrum antibacterial activity [29].

Phyllanthus niruri

P. niruri has many effective traditional uses for a wide variety of diseases. Some of the medicinal uses have the plant extracts possess in various pharmacological properties [40]. *Phyllanthus* have been informed in medicinal properties that used to treat hypertension, jaundice, diabetes, hypercalciuria, and urolithiasis [40]. This species also has demonstrated an antimutagenic and anticarcinogenic action, antitumor, antioxidant, hepatoprotective and antihyperuricemic properties, as well as antihyperlipemic activity. The study of "Growth

inhibitory effects of *P. niruri* extracts in with cisplatin on cancer cell lines" showed that combinations of plant extracts and chemotherapeutic agents may allow for a reduction in the dosage of the more toxic chemotherapeutic agent while retaining the therapeutic efficacy and minimizing toxicities [40]. Moreover, the induction of cell death by SDEPN may be a strategy for increasing the sensitivity of HT29 cells to cisplatin-mediated cell death [40].

Tinospora cordifolia

In traditional system of medicine, *T. Cordifolia* (Willd.) has been use this herb for phytochemical, pharmacological and clinical investigations and it showe the medical properties on immunomodulation, anticancer activity, liver disorders, antidiarrhoeal, anti-oxidant activity, aphrodisiac activity, anthelmintic activity, antipsychotic activity, and hypoglycemic [41]. The study showed result that the extract of *Tinospora cordifolia* exhibits inhibition zone on limited species such like *S. aureus* (12 mm), *Klebsiella pneumonia* (10 mm), *Pseudomonas sp* (8 mm), *Aspergillus niger* (6 mm), *A. fumigates* (8 mm) *and Mucor sp* (12 mm) [41]. So that, it is expected that using *T.cordifolia* as therapeutic agents for treating infections in traditional medicine [4].

METHODOLOGY

Preparation of culture

E. coli ATCC25822, *S. enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), *S. enterica* 4,5,12: i-(human) US clone, *B. cereus*, and *L. monocytogenes* 10403S cultures (one loop for each) were added onto 50ml of individual fresh media (nutrient broth (NB) and Brain Hearth Infusion (BHI) broth for *L. monocytogenes* 10403S and incubated at 37°C for night. Then, 1%(v/v) of overnight culture was inoculated into 50 ml of fresh NB for *E. coli* ATCC25822, *S. enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), *S. enterica* 4,5,12: i-(human) US clone, and *B. cereus*. And 50 ml of fresh BHI for *L. monocytogenes* 10403S, at 37°C by shaker incubator until OD_{600} reach 0.1 (SPECTRONIC, model GENESYS 5) which is early log phase [26],[27].

Plant sample preparation

Seven herb samples; *T. spathacea*, *A. paniculata*, *C. nutans*, *E. acicularis*, *A.concinna*, *P. niruri*, and *T. cordifolia*, which are collected from locality at Pakthongchai prefecture, Nakhonratchasima provience, Thailand.

All fresh herbs were use in whole plant. First, herbs were clean and reduce the size into small pieces. Then, herbs were stored in refrigerator at 6°C until use.

Extraction

The 20 g of each herb was weighed on Top-loaded balance with 1 decimal, using ZEPPER model ES-300. Then, herbs was added into 60 ml of five extract solvents (95% Ethanol, Sterile distilled water, Sterile distilled water (autoclaving at 121°C 15 PSI for 15 minutes using HICLAVETM model HA-300 MII), Chloroform and Hexane) which individually extracted. The herbs were soaked for 48 hours at room temperature with stirring every 12

hours. Then, supernatant were filtered through cheesecloth and centrifuged by using Chermle medel Z230A, at 5000 rpm for 5 minutes. The clear supernatant was collected in 100 ml beaker and dried in water bath by using Schutzart DIN40050-IP20, at 45°C until the clear solution become condense or slurry. All crude extracts were kept in freezer at -20°C until use [26],[27].

The crude extract was diluted 10% (v/v) by extract solvent to solvent. Then, diluted crude extracts were kept in freezer at -20°C.

Antibacterial Assay

Media Preparation

Fresh nutrient broth (NB) was prepared by mixing 13 g of Nutrient Broth, HIMEDIA, Dehydrate culture media with 1000 ml of water and mixed well. For nutrient agar, 13 g of Nutrient Broth (HIMEDIA), Dehydrate culture media was mixed with 15 g of agar powder in 1000 ml of water.

Fresh Brain Heart Infusion (BHI) was used and prepared by mixing 37 g of Brain Heart Infusion Broth (CRITERION), Dehydrate culture media with 1000 ml of water and mixed well. For nutrient agar, 37 g of Brain Heart Infusion Broth (CRITERION), Dehydrate culture media was mixed with 15 g of agar powder in 1000 ml of water.

These four medias have to sterile by autoclave at 121°C 15 PSI for 15 minutes using HICLAVETM model HA-300 MII before use.

For Salmonella-Shigella agar (SS) was prepared by mixed 63.02 g of Salmonella-Shigella Agar (HIMEDIA) with 1000 ml of water and mixed well, using microwave to melt the media until it change to be the liquid form [26],[27].

Disk diffusion method

The 100 µl of culture was swapped on nutrient agar for E.coli and B. cereus, SS agar for *S.enterica* Typhimurium U302 (DT1046), *S.enterica* Enteritidis (human), *S. enterica* 4,5,12: i-(human) US clone, and BHI agar for *L. monocytogenes* 10403S. Four Filter paper discs (Whatman filter paper) of 6 mm diameter were prepared and sterilized. The first disc was added 15 µl of Penicillin-G(Fluka BilChemika) (100 mg/ml) as positive control, second disc was added 15 µl of solvent (95% Ethanol, Sterile distilled water, Sterile distilled water for auto claving at 121°C 15 PSI for 15 minutes, Chloroform and Hexane) as negative control, and other two discs were added 15 µl of herb crude extract The plates were incubated at 37°C for 24 hours with an upright position. The diameters of clear zone were measured in mm the data were collected, which mean and standard deviation of data were also calculated by using Microsoft Excel 2007 [26],[27]. The experiments were done 3 times independently duplicate.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The broth dilution method was used for the MIC determination. The 0, 32, 64, 128, and 256 µl crude extracts were added into 1 ml the fresh broth. Then 100µl of culture with 0.1 OD600 was inoculated into each tube. The tubes which show the negative result in MIC test that can inhibit growth of bacteria cell were chosen in MBC test. The MIC negative result tubes were mixed well and one loop of broth was streaked on agar plate media. All plates were incubated at 37°C for 24 hours. The growth of microbe was observed, the MBC value is the concentration of antibiotic that can kill most microbes in media [26],[27].

RESULTS

Part I: Antibacterial Assay

1. *E. coli* ATCC25822

The results in table 1 showed that the extraction condition affect the antibacterial activity. The 95% ethanol extraction condition gave the best antibacterial activity in all herbs against *E. coli* ATCC25822 as showed in figure 1. The *C. nutans* 95% ethanol crude extract, gave the highest antibacterial activity; 8.5±0.58 mm. The *A. paniculata and A. concinna* 9 5% ethanol crude extract gave the lowest antibacterial activity against 7.25±0.50 mm. For Chloroform crude extract, the antibacterial activity was ranged from 8.25±0.50 to no inhibition. The positive control for 95% Ethanol, Chloroform and Penicillin-G is showed the zone of inhibition of antibacterial activity which is 8.83±0.75 mm, 8.9±0.66 mm and 44±0.91 mm, respectively.

Table 1: The antibacterial activity (clear zone in mm) of seven herbs extracted under different extractions condition against *E. coli* ATCC25822

Herb extraction	Penicillin-G	95% Ethanol	Sterile Distilled water	Autoclaving at 121°C 15 PSI for 15 minutes	Hexane	Chloroform
T. spathacea		7.75±0.96	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
A. paniculata		7.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C. nutans		8.5±0.58	0.00±0.00	0.00±0.00	0.00±0.00	8.25±0.50
E. acicularis		8.0± 1.15	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
A. concinna		7.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	7.25±0.50
P. niruri		8.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T. cordifolia		7.75±0.96	0.00±0.00	0.00±0.00	0.00±0.00	7.25±0.50
Solvent control	45.09±1.15	8.83±0.75	0.00±0.00	0.00±0.00	0.00±0.00	8.9±0.66

The MIC and MBC results were showed in Table 2. The MIC and MBC of *E. acicularis*, *A. concinna*, *P. niruri* 95% ethanol crude extract showed 64 µl/ml. While the MIC and MBC of *T. spathacea*, *A. paniculata*, *C. nutans*, *T. cordifolia* 95% ethanol crude extract showed 128 µl/ml. The MIC and MBC of *A. concinna*, and *T. cordifolia* chloroform crude extract showed 32 µl/ml. While the MIC and MBC of chloroform crude extract *C. nutans*, showed 32 µl/ml and 128 µl/ml, respectively.

Table 2: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on seven herbs extracted under different extractions condition against on *E. coli* ATCC25822

Extraction method	Herb extracted	MIC (µl/ml)	MBC (µl/ml)
95% Ethanol	*T. spathacea*	128	128
	A. paniculata	128	128
	C. nutans	128	128
	E. acicularis	64	64
	A. concinna	64	64
	P. niruri	64	64
	T. cordifolia	128	128
Chloroform	*C. nutans*	32	128
	A. concinna	32	32
	T. cordifolia	32	32

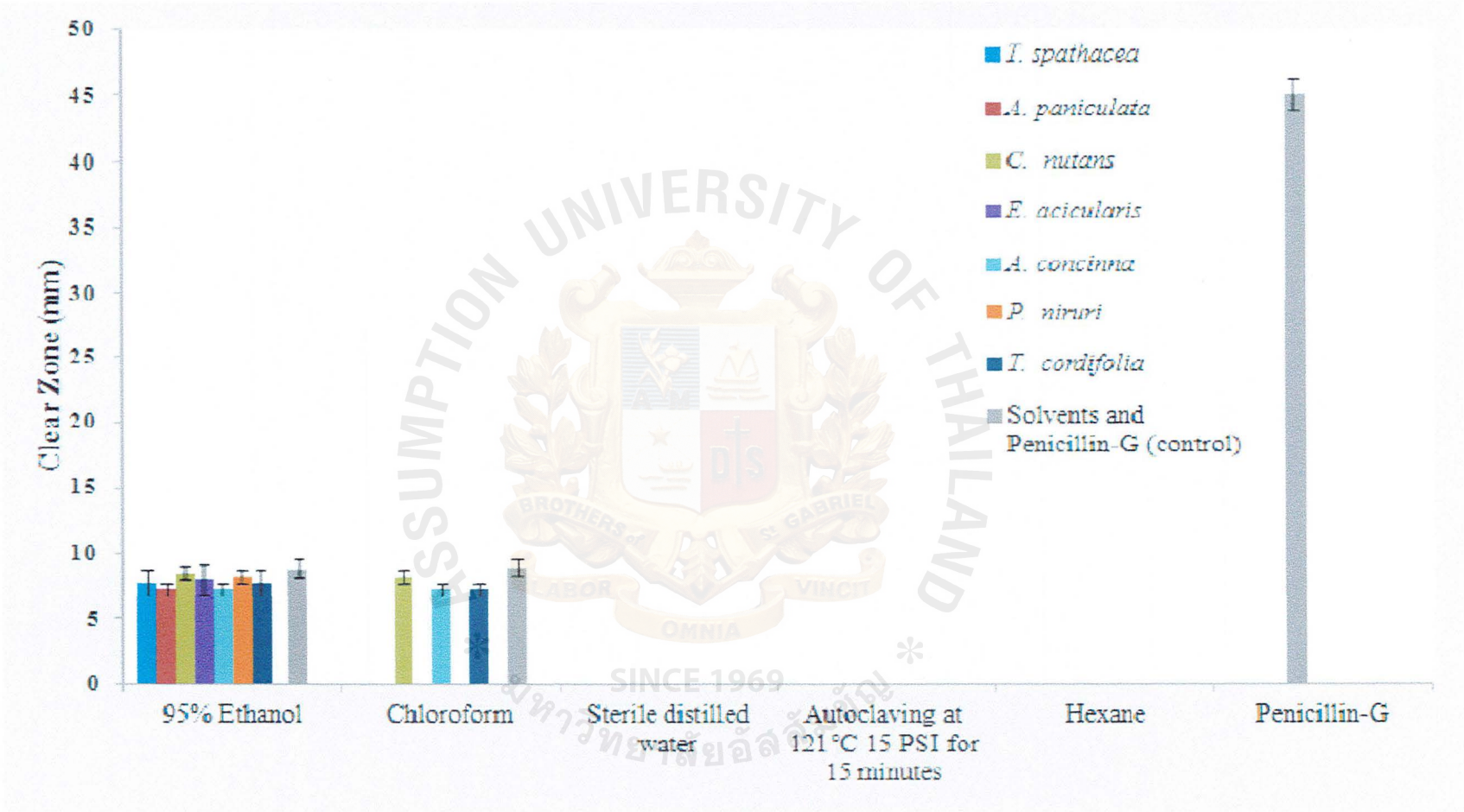

Figure 1: Antibacterial activity as clear zone of herbs extracted under different extractions condition against on *E. coli* ATCC25822

2. *S. enterica* Typhimurium U302 (DT1046)

The results in table 3 showed that the extraction condition affect the antibacterial activity. The chloroform extraction condition gave the best antibacterial activity in all herbs against *S. enterica* Typhimurium U302 (DT1046) as showed in figure 2. The 95% ethanol, *E. acicularis* extract, gave the highest antibacterial activity; 9.75±2.22 mm. For the sterile distilled water extraction condition, the inhibition of antibacterial activity was ranged from 7.75±0.96 to 7.75±0.50 mm. For Chloroform crude extract, the antibacterial activity was ranged from 11.50±1.29 to 9.50±1.73 mm. *C. nutans* is showed the highest antibacterial activity and diameter for zone of inhibition is 11.50±1.29 mm. The positive control for 95% ethanol, sterile distill water, chloroform and Penicillin-G is showed the zone of inhibition of antibacterial activity which is 10.21±0.89mm, 7.75±0.71mm, 10.86±1.1mm, and 17±1.44mm, respectively.

Table 3: The antibacterial activity (clear zone in mm) of seven herbs extracted under different extractions condition against on *S. enterica* Typhimurium U302 (DT1046)

Herb extraction	Penicillin-G	95% Ethanol	Sterile Distilled water	Autoclaving at 121°C 15 PSI for 15 minutes	Hexane	Chloroform
T. spathacea		9.25±0.5	0.00±0.00	0.00±0.00	0.00±0.00	10.50±1.29
A. paniculata		8.25±0.96	0.00±0.00	0.00±0.00	0.00±0.00	9.50±1.73
C. nutans		7.50±0.58	0.00±0.00	0.00±0.00	0.00±0.00	11.50±1.29
E. acicularis		9.75±2.22	0.00±0.00	0.00±0.00	0.00±0.00	10.50±1.91
A. concinna		8.75±2.06	0.00±0.00	0.00±0.00	0.00±0.00	10.00±0.82
P. niruri		7.50±0.58	7.75±0.50	0.00±0.00	0.00±0.00	10.25±1.50
T. cordifolia		9.00±1.41	7.75±0.96	0.00±0.00	0.00±0.00	10.50±2.08
Solvent control	17±1.44	10.21±0.89	7.75±0.71	0.00±0.00	0.00±0.00	10.86±1.1

The MIC and MBC results were showed in Table 4. The MIC and MBC of *E. acicularis* and *P. niruri* showed 64 µl/ml, *T. spathacea, A. paniculata, C. nutans, and T. cordifolia* showed 128 µl/ml and *A. concinna* showed 256 µl/ml. The extraction of sterile distilled water, *P. niruri* and *T. cordifolia* showed 256 µl/ml for MIC and >256 µl/ml for MBC test. For the extraction of chloroform, The MIC and MBC of *E. acicularis* and *C. nutans* showed 32 µl/ml, other five herbs extracted showed 64 µl/m.

Table 4: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on seven herbs extracted under different extractions condition against on *S. enterica* Typhimurium U302 (DT1046)

Extraction method	Herb extracted	MIC (µl/ml)	MBC (µl/ml)
95% Ethanol	*T. spathacea*	128	128
	A. paniculata	128	128
	C. nutans	128	128
	E. acicularis	64	64
	A. concinna	256	256
	P. niruri	64	64
	T. cordifolia	128	128
Sterile distilled water	*P. niruri*	256	>256
	T. cordifolia	256	>256
Chloroform	*T. spathacea*	64	64
	A. paniculata	64	64
	C. nutans	32	32
	E. acicularis	32	32
	A. concinna	64	64
	P. niruri	64	64
	T. cordifolia	64	64

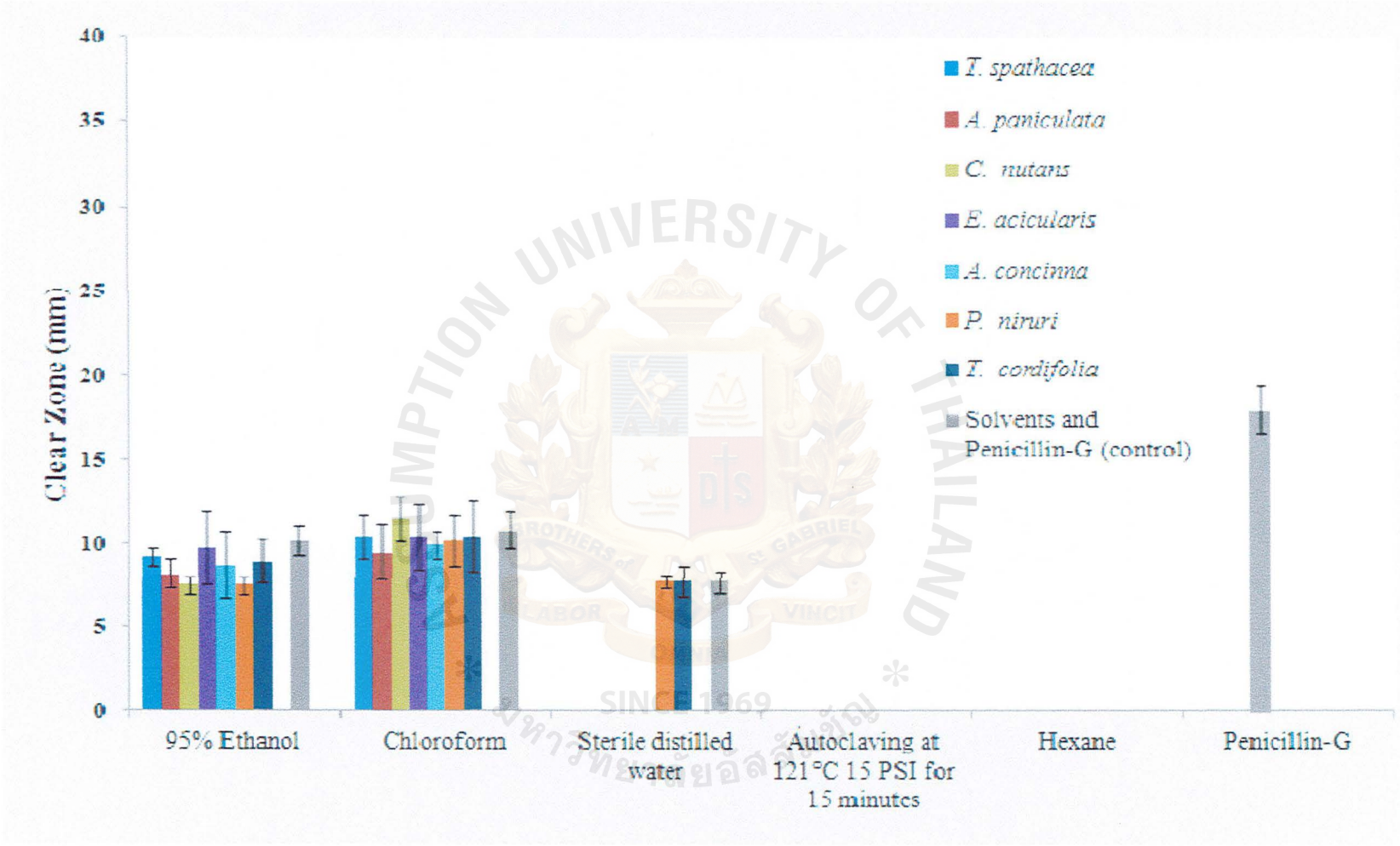

Figure 2: Antibacterial activity as clear zone of herbs extracted under different extractions condition against on *S. enterica* Typhimurium U302 (DT1046)

3. *S. enterica* Enteritidis (human)

The results in table 5 showed that the extraction condition affect the antibacterial activity. The chloroform extraction condition gave the best antibacterial activity in all herbs against *S. enterica* Enteritidis (human) as showed in figure 3. The 95% ethanol extraction condition, only *T. spathacea* and *A. paniculata* showed the antibacterial activity. For the extraction of Chloroform, the inhibition of antibacterial activity was ranged from 10.00±1.63 to 8.50±1.29 mm. *C. nutans* showed the highest antibacterial activity is 10.25±0.96 mm. The positive control for 95% ethanol, chloroform and Penicillin-G is showed the zone of inhibition of antibacterial activity which is 20.93±1.44mm, 11.29±1.07mm, and 37.49±1.52mm, respectively.

Table 5: The antibacterial activity (clear zone in mm) of seven herbs extracted under different extractions condition against on *S. enterica* Enteritidis (human)

Herb extraction	Penicillin-G	95% Ethanol	Sterile Distilled water	Autoclaving at 121°C 15 PSI for 15 minutes	Hexane	Chloroform
T. spathacea		8.75±0.96	0.00±0.00	0.00±0.00	0.00±0.00	8.75±1.71
A. paniculata		7.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	8.50±1.29
C. nutans		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.25±0.96
E. acicularis		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.00±1.63
A. concinna		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	8.25±0.50
P. niruri		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	9.25±1.71
T. cordifolia		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	9.25±2.06
Solvent control	37.49±1.52	20.93±1.44	0.00±0.00	0.00±0.00	0.00±0.00	11.29±1.07

The MIC and MBC results were showed in Table 6. MIC and MBC of *T. spathacea* and *A. paniculata* extracted showed 128 µl/ml for extraction of 95% ethanol extract. For the extraction of chloroform, The MIC and MBC of *T. spathacea*, *C. nutans*, *E. acicularis*, *A. concinna*, and *P. niruri* showed 64 µl/ml. For *A. paniculata* and *T. cordifolia* showed 32 µl/ml.

Table 6: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on seven herbs extracted under different extractions condition against on *S. enterica* Enteritidis (human)

Extraction method	Herb extracted	MIC (µl/ml)	MBC (µl/ml)
95% Ethanol	*T. spathacea*	128	128
	A. paniculata	128	128
Chloroform	*T. spathacea*	32	32
	A. paniculata	64	64
	C. nutans	32	32
	E. acicularis	32	32
	A. concinna	32	32
	P. niruri	32	32
	T. cordifolia	64	64

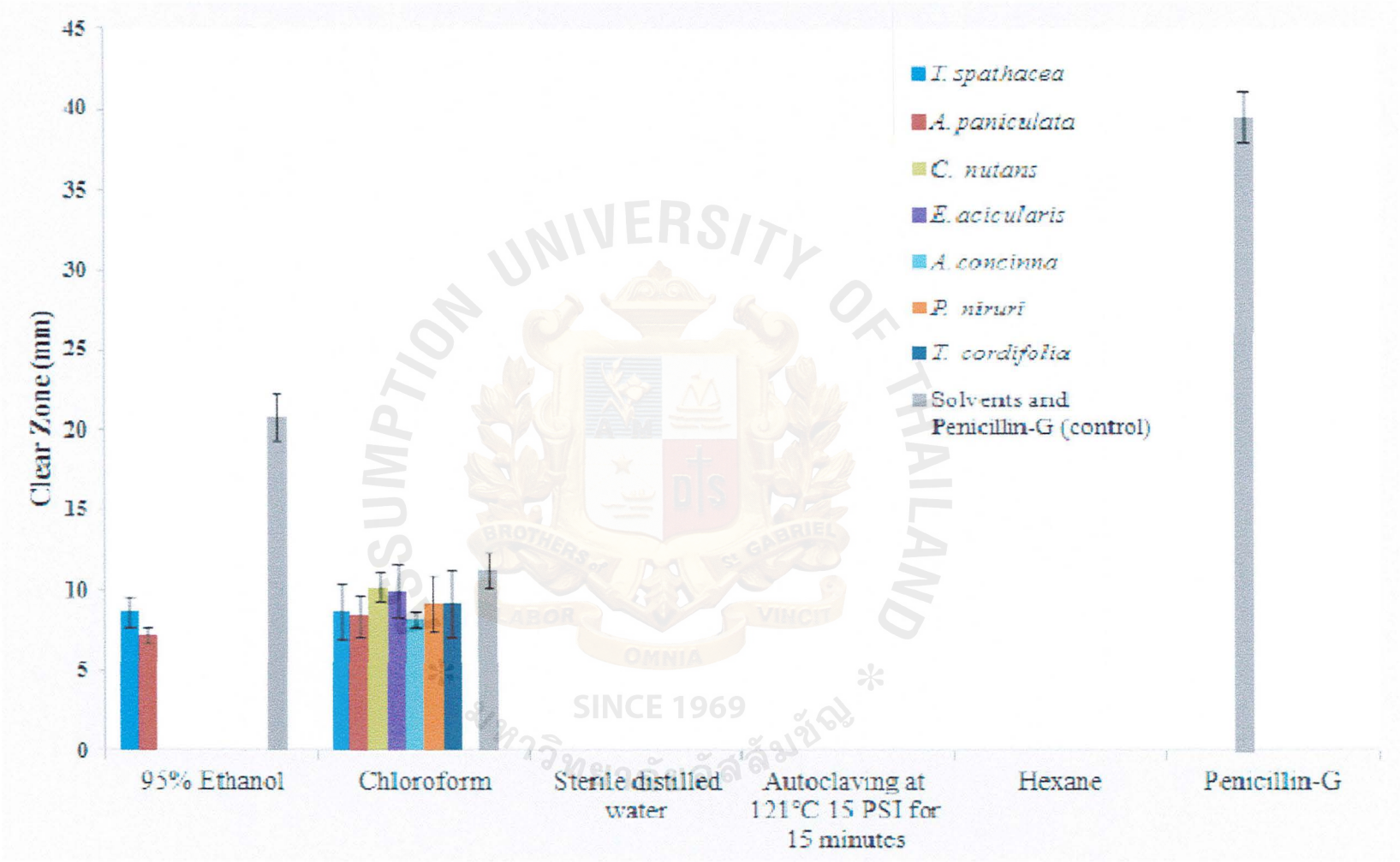

Figure 3: Antibacterial activity as clear zone of herbs extracted under different extractions condition against on *S. enterica* Enteritidis (human)

4. *S.enterica* 4,5,12: i-(human) US clone

The results in table 7 showed that the extraction condition affect the antibacterial activity. The chloroform extraction condition gave the best antibacterial activity in all herbs against *S. enterica* 4,5,12: i-(human) US clone as showed in figure 4. For the extraction of 95% ethanol, *A. concinna* showed higher antibacterial activity is 10.5±1.29 mm. and *E. acicularis* showed the lower antibacterial activity is 7.75± 0.96 mm, respectively. For chloroform extraction condition, the inhibition of antibacterial activity was ranged from 11.00±1.41 to 8.50±0.58 mm. *C. nutans* showed the highest antibacterial activity is 11.00±1.41 mm. The positive control for 95% ethanol, chloroform and Penicillin-G is showed the zone of inhibition of antibacterial activity which is 8.83±0.75 mm, 9.5±1.22 mm, and 25.63±1.22 mm, respectively.

Table 7: The antibacterial activity (clear zone in mm) of seven herbs extracted under different extractions condition against on *S. enterica* 4,5,12: i-(human) US clone

Herb extraction	Penicillin-G	95% Ethanol	Sterile Distilled water	Autoclaving at 121°C 15 PSI for 15 minutes	Hexane	Chloroform
T. spathacea		9.25±1.5	0.00±0.00	0.00±0.00	0.00±0.00	9.25±0.96
A. paniculata		9.25±1.26	0.00±0.00	0.00±0.00	0.00±0.00	10.75±0.96
C. nutans		8.25±0.96	0.00±0.00	0.00±0.00	0.00±0.00	11.00±1.41
E. acicularis		7.75± 0.96	0.00±0.00	0.00±0.00	0.00±0.00	8.50±0.58
A. concinna		10.5±1.29	0.00±0.00	0.00±0.00	0.00±0.00	9.25±0.96
P. niruri		8.75±0.96	0.00±0.00	0.00±0.00	0.00±0.00	9.75±0.96
T. cordifolia		8.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	9.25±1.26
Solvent control	25.63±1.22	8.83±0.75	0.00±0.00	0.00±0.00	0.00±0.00	9.5±1.22

The MIC and MBC results were showed in Table 8. The MIC and MBC of all seven herbs extracted showed 128 µl/ml extraction of 95% ethanol. For the extraction of chloroform, MIC and MBC of *A. paniculata* and *P. niruri* showed 32 µl/ml, other five herbs extracted showed 64 µl/ml.

Table 8: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on seven herbs extracted under different extractions condition against on *S. enterica* 4,5,12: i-(human) US clone.

Extraction method	Herb extracted	MIC (µl/ml)	MBC (µl/ml)
95% Ethanol	*T. spathacea*	128	128
	A. paniculata	128	128
	C. nutans	128	128
	E. acicularis	128	128
	A. concinna	128	128
	P. niruri	128	128
	T. cordifolia	128	128
Chloroform	*T. spathacea*	64	64
	A. paniculata	32	32
	C. nutans	64	64
	E. acicularis	64	64
	A. concinna	64	64
	P. niruri	32	32
	T. cordifolia	64	64

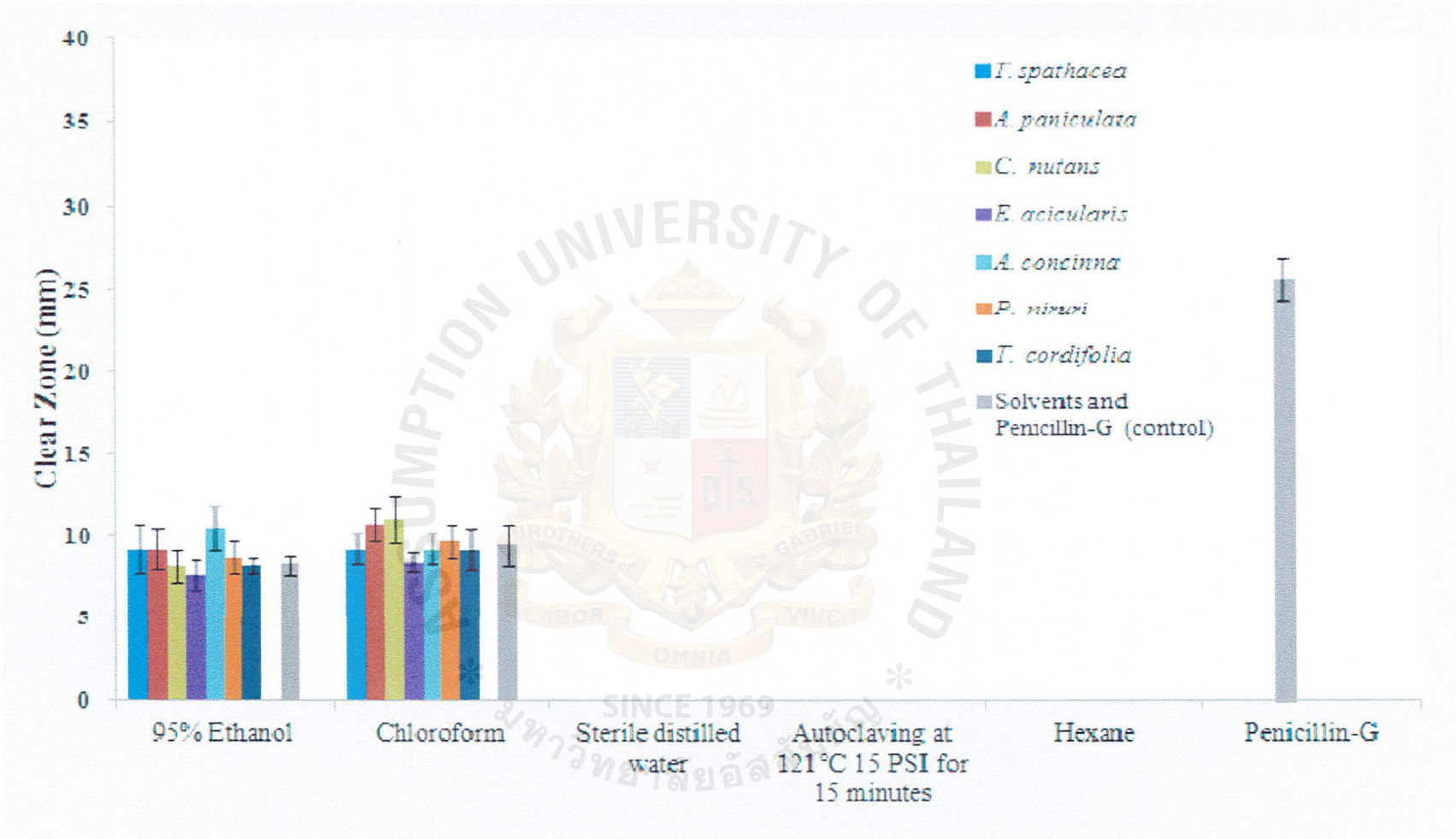

Figure 4: Antibacterial activity as clear zone of herbs extracted under different extractions condition against on *S. enterica* 4,5,12: i-(human) US clone

5. *Bacillus cereus*

The results in table 9 showed that the extraction condition affect the antibacterial activity. For the 95% ethanol extraction condition, *E. acicularis* showed highest antibacterial activity is 8.50± 0.58 mm. For chloroform extraction condition, *T. cordifolia is* showed the highest antibacterial activity is 8.75±1.26 mm. The positive control for 95% ethanol, chloroform and Penicillin-G is showed the zone of inhibition of antibacterial activity which is 8.21±0.89 mm, 8.14±0.66 mm, and 19.71±1.04 mm, respectively.

Table 9: The antibacterial activity (clear zone in mm) of seven herbs extracted under different extractions condition against on *B. cereus*

Herb extraction	Penicillin-G	95% Ethanol	Sterile Distilled water	Autoclaving at 121°C 15 PSI for 15 minutes	Hexane	Chloroform
T. spathacea		8.00±0.82	0.00±0.00	0.00±0.00	0.00±0.00	8.00±0.82
A. paniculata		8.00±0.82	0.00±0.00	0.00±0.00	0.00±0.00	8.25±0.50
C. nutans		7.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	8.75±0.50
E. acicularis		8.50± 0.58	0.00±0.00	0.00±0.00	0.00±0.00	8.00±0.82
A. concinna		7.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	7.50±0.58
P. niruri		7.50±0.58	0.00±0.00	0.00±0.00	0.00±0.00	8.25±0.50
T. cordifolia		7.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	8.75±1.26
Solvent control	19.71±1.04	8.21±0.89	0.00±0.00	0.00±0.00	0.00±0.00	8.14±0.66

The MIC and MBC results were showed in Table 10. The MIC of all seven herbs extracted showed 128 µl/ml. The MBC, all of seven herbs extracted showed 256 µl/ml for extraction of 95% ethanol. For the extraction of chloroform, The MIC of all seven herbs extracted showed 32 µl/ml, the MBC of *T. spathacea* and *A. paniculata* showed 32 µl/ml and five herbs extracted showed 256 µl/ml.

Table 10: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on seven herbs extracted under different extractions condition against on *B. cereus*

Extraction method	Herb extracted	MIC (µl/ml)	MBC (µl/ml)
95% Ethanol	*T. spathacea*	128	256
	A. paniculata	128	256
	C. nutans	128	256
	E. acicularis	128	256
	A. concinna	128	256
	P. niruri	128	256
	T. cordifolia	128	256
Chloroform	*T. spathacea*	32	32
	A. paniculata	32	32
	C. nutans	32	256
	E. acicularis	32	256
	A. concinna	32	256
	P. niruri	32	256
	T. cordifolia	32	256

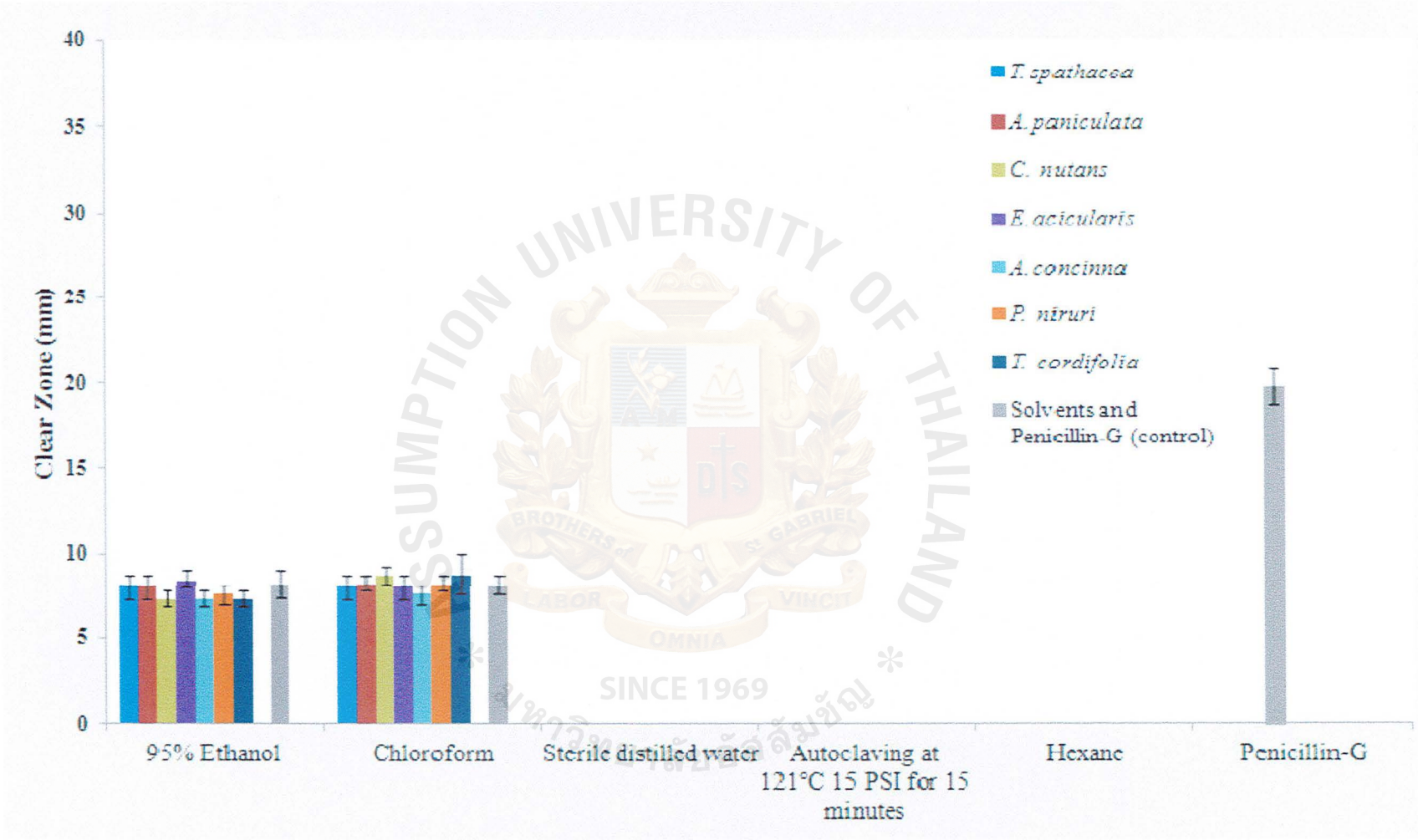

Figure 5: Antibacterial activity as clear zone of herbs extracted under different extractions condition against on *B. cereus*

6. *Listeria monocytogenes* 10403S

The results in table 9 showed that the extraction condition affect the antibacterial activity. The chloroform extraction condition gave the best antibacterial activity in all herbs against *S. enterica* 4,5,12: i-(human) US clone as showed in figure 6. For 95% ethanol extraction condition, *E. acicularis* showed highest antibacterial activity is 8.50± 1.00 mm. For chloroform extraction condition, *P. niruri* is showed the highest antibacterial activity is 10.00±1.41 mm. The positive control for 95% ethanol, sterile distilled water, sterile distilled water for Autoclaving, chloroform and Penicillin-G is showed the zone of inhibition of antibacterial activity which is 8.60±1.5 mm, 7.21±0.80 mm, 7.43±0.85 mm, 8.43±0.94 mm, and 19.83±1.65 mm, respectively.

Table 11: The antibacterial activity (clear zone in mm) of seven herbs extracted under different extractions condition against on *L. monocytogenes* 10403S

Herb extraction	Penicillin-G	95% Ethanol	Sterile Distilled water	Autoclaving at 121°C 15 PSI for 15 minutes	Hexane	Chloroform
T. spathacea		6.25±0.50	0.00±0.00	0.00±0.00	0.00±0.00	8.75±0.96
A. paniculata		6.50±0.58	0.00±0.00	0.00±0.00	0.00±0.00	8.25±2.06
C. nutans		7.25±0.96	0.00±0.00	0.00±0.00	0.00±0.00	9.25±1.50
E. acicularis		8.50± 1.00	0.00±0.00	0.00±0.00	0.00±0.00	9.25±0.96
A. concinna		7.25±0.96	0.00±0.00	0.00±0.00	0.00±0.00	9.25±0.96
P. niruri		8.25±1.50	0.00±0.00	0.00±0.00	0.00±0.00	10.00±1.41
T. cordifolia		7.00±0.82	0.00±0.00	0.00±0.00	0.00±0.00	8.75±0.96
Solvent control	19.83±1.65	8.60±1.5	7.21±0.80	7.43±0.85	0.00±0.00	8.43±0.94

The result of MIC and MBC were showed in Table 12. The MIC and MBC of all seven herbs extracted showed 128 µl/ml for extraction of 95% ethanol. The extraction of chloroform, MIC of all seven herbs extracted showed 32 µl/ml, the MBC of *A. paniculata* and *C. nutans* showed 32 µl/ml, *T. spathacea* showed 64 µl/ml, *T. cordifolia* showed 128 µl/ml and other three herbs extracted showed 256 µl/ml.

Table 12: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on seven herbs extracted under different extractions condition against on *L. monocytogenes* 10403S

Extraction method	Herb extracted	MIC (µl/ml)	MBC (µl/ml)
95% Ethanol	*T. spathacea*	128	128
	A. paniculata	128	128
	C. nutans	128	128
	E. acicularis	128	128
	A. concinna	128	128
	P. niruri	128	128
	T. cordifolia	128	128
Chloroform	*T. spathacea*	32	64
	A. paniculata	32	32
	C. nutans	32	32
	E. acicularis	32	256
	A. concinna	32	256
	P. niruri	32	256
	T. cordifolia	32	128

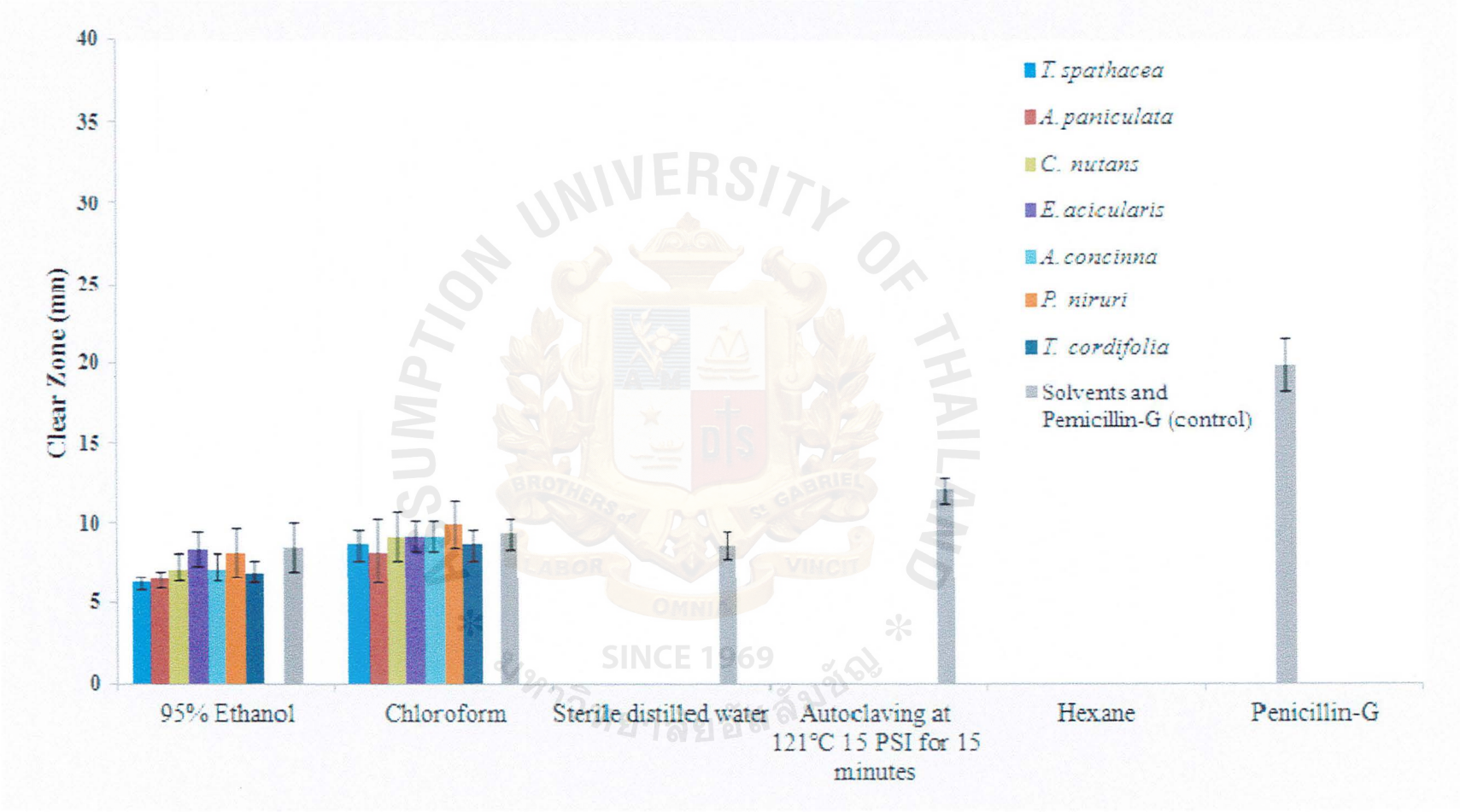

Figure 6: Antibacterial activity as clear zone of herbs extracted under different extractions condition against on *L. monocytogenes* 10403S

DISCUSSION

From figure 1-6, the results showed the antibacterial activity mostly in 95% ethanol and chloroform extraction condition in all seven herbs; *T. spathacea, A. paniculata, C. nutans, E. acicularis, A. concinna , P. niruri, and T. cordifolia.* The active compounds that can extract from herbs by different extraction conditions were indicated in the result.

For *T. spathacea,* only 95%Ethanol and Chloroform extraction conditions showed the antibacterial activity against all bacteria, while other three extraction conditions had no antibacterial activity of *T. spathacea.* Its antibacterial activity aginst the growth both positive and negative bacterial that used in this experiment which are *S. enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), *S. enterica* 4,5,12: i-(human) US clone, *B. cereus*, and *L. monocytogenes* 10403S except for *E. coli* ATCC25822. The highest inhibition is on *S. enterica* 4,5,12: i-(human) US clone which diameter of clear zone is 9.25±1.5 mm for 95%Ethanol extraction condition and *S. enterica* Typhimurium U302 (DT1046) which diameter of clear zone is 10.50±1.29 for Chloroform extraction condition, respectively. In addition for the MIC and MBC determination, the crude extract can inhibit all bacteria with 128 µl/ml of MIC test. For MBC, *E. coli* ATCC25822, *S. enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), and *S. enterica* 4,5,12: i-(human) US clone were kill by 64 µl/ml which is highest antibacterial effect for 95%Ethanol extraction condition. For the chloroform extraction condition, the crude extract can inhibit and kill *S. enterica* Enteritidis (human) and *B. cereus* in minimum concentration which is 32 µl/ml. *T. spathacea* extracted contained phenolic compounds, tannin and flavonoid [17]. The other study reported that flavonoids can extract by using methanol extract, cadiac glycosides can extract by using 70% ethanol, tannins can extract by using extract leaves with ethanol, and terpenoids can extract by powder leaves extract with methanol and water. Moreover, the *T. spathacea* extracted have no antibacterial activity against *Staphylococcus saprophyticus* (ATCC 15305), *S. aureus* (ATCC 6341), *Escherichia coli* (ATCC 4157), *Haemophilus influenzae* (ATCC 8142), *Pseudomonas aeruginosa* (ATCC 7700) *and Proteus vulgaris* (ATCC 6896) with aqueous crude extract [19].

A. paniculata, only 95%Ethanol and Chloroform extraction conditions showed the antibacterial activity against all bacteria, while other three extraction conditions had no presented the antibacterial activity of *A. paniculata.* This antibacterial activity against all six

bacteria for 95%Ethanol extraction condition but the Chloroform extraction condition cannot against only the growth of *E. coli* ATCC25822, the highest antibacterial activity is on *S. enterica* 4,5,12: i-(human) US clone which diameter of clear zone is 9.25±1.26 mm for 95%Ethanol extraction condition and 10.75±0.96 mm for chloroform extraction condition, respectively. In addition for the MIC and MBC determination, the crude extract can inhibit *E. coli* ATCC25822, *S. enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), and *S. enterica* 4,5,12: i-(human) US clone were inhibit by 128 µl/ml and kill by 64 µl/ml under 95%Ethanol extraction condition. For the chloroform extraction condition, the crude extract can inhibit and kill *S. enterica* 4,5,12: i-(human) US clone, *B. cereus*, and *L. monocytogenes* 10403S in minimum concentration which is 32 µl/ml. *A. paniculata* extracted contains diterpenes, lactones, and flavonoids (mainly exist in the root) [20]. The active componds that can extract from methanol extract included andrographolide, neo-andrographolide, 14-deoxy-andrographolide, diterpenoids, flavonoids, and polyphenol [21]. The other study report that the water extract of boiling roots have effective against *S. aureus*. For methanol extract of stem have antibacterial activity against *Proteus vulgaris*, stem and leaves powder extract have antibacterial activity against *Shigella* bacteria but it is not effective against cholera. The ethanol extract of leaves have antibacterial activity against *S. aureus* and *E. coli* [22]. The study of ethanol extract of *A. paniculata* powder extracted is effective against *B. cereus* and *L. monocytogenes* [26].

C. nutans, the results of this experiment indicated that the *C. nutans* chloroform crude extracted showed antibacterial activity against all six bacteria that used in this experiment. While *C. nutans* 95% ethanol crude extracted can inhibit five bacteria except for *S. enterica* Enteritidis (human). For other three extraction conditions had no present the antibacterial activity of *C. nutans* extracted. The highest antibacterial activity is on *S. enterica* 4,5,12: i-(human) US clone which diameter of clear zone is 8.25±0.96 mm for 95%Ethanol extraction condition and 11.00±1.41 mm for Chloroform extraction condition, repectively. In addition for the MIC and MBC determination, the crude extract can inhibit *E. coli* ATCC25822, *S. enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), and *L. monocytogenes* 10403S were inhibit by 128 µl/ml and kill by 64 µl/ml for 95%Ethanol extraction condition. For the chloroform extraction condition, the crude extract can inhibit and kill *S. enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), and *L. monocytogenes* 10403S in minimum concentration which is 32 µl/ml. In *C. nutans* extracted contained "C-glycosyl flavones, vitexin, isovitexin, shaftoside, isomollupentin 7-O-

β-glucopyranoside, orientin, isoorientin and sulfur containing glucosides" have been isolated from the extraction condition of the stem and leaves which had collected in Thailand [5]. The eight compounds that extracted from *C. nutans* were relates to chlorophyll a and chlorophyll b which are 13^2-hydroxy-(13^2-S)-chlorophyll b, 13^2-hydroxy-(13^2-R)-chlorophyll b, 13^2-hydroxy-(13^2-S)-phaeophytin b, 13^2-hydroxy-(13^2-R)-phaeophytin b, 13^2-hydroxy-(13^2-S)-phaeophytin a, 13^2-hydroxy-(13^2-R)-phaeophytin a, purpurin 18 phytyl ester, and phaeophorbide a. These compounds are shown the anti-herpes simplex activity [6]. The other study of this plant with methanol extract the result showed that it is effective against *S. aureus, E. Coli, P. acnes, S. epidermidis,* and *B. cereus* with active compounds; C-glycosyl flavones, Vitexin, Isovitexin, Shaftoside, Isomollupentin, 7-O-β-glucopyranoside, Orientin, Isoorientin, and Glucosides [7].

E. acicularis, the results of this experiment indicated that the only *E. acicularis* 95% ethanol and chloroform crude extracted showed antibacterial activity against five bacteria that used in this experiment excepted for *S. enterica* Enteritidis (human) and *E. coli* ATCC25822, respectively. For other three extraction conditions had no present the antibacterial activity of *E. acicularis* extracted. The highest antibacterial activity against *S. enterica* Typhimurium U302 (DT1046) which diameter of clear zone is 9.75±2.22 mm for 95%Ethanol extraction condition and 10.50±1.91 mm for Chloroform extraction condition, respectively. In addition for the MIC and MBC determination, the crude extract can inhibit *E. coli* ATCC25822 and *S. enterica* Typhimurium U302 (DT1046 were inhibit and kill in low concentration which is 64 µl/ml for 95%Ethanol extraction condition. For the chloroform extraction condition, the crude extract can inhibit and kill *S. enterica* Typhimurium U302 (DT1046) and *S. enterica* Enteritidis (human) in minimum concentration which is 32 µl/ml. *Eleocharis* sp. contained "a high concentration of β-sitosterol and lupeol but the most dominant component was β-sitostanol (24-ethylcholestan-3β-ol)" [23]. This plant have no report on antibacterial activity. It means that β-sitosterol and lupeol have antibacterial capacity to against most of bacteria that used in this experiment.

A. concinna, the results of this experiment indicated that the *E. acicularis* 95% Ethanol crude extracted showed antibacterial activity against five bacteria excepted for *S. enterica* Enteritidis (human) and *A. concinna* chloroform extracted showed antibacterial activity against all bacteria used in this experiment. For other three extraction conditions had no present the antibacterial activity of *A. concinna* extracted. The highest antibacterial activity

against *S. enterica* 4,5,12: i-(human) US clone which diameter of clear zone is 10.5±1.29 mm for 95%Ethanol extraction condition and on *S. enterica* Typhimurium U302 (DT1046) which diameter of clear zone is 10.00±0.82 mm for Chloroform extraction condition, respectively. In addition for the MIC and MBC determination, the crude extract can inhibit *E. coli* ATCC25822 was inhibit and kill in low concentration which is 64 µl/ml for 95%Ethanol extraction condition. For the chloroform extraction condition, the crude extract can inhibit and kill *E. coli* ATCC25822 and *S. enterica* Enteritidis (human) in minimum concentration which is 32 µl/ml. In previous study, the crude extracted of *A. concinna* pod can inhibit *B. subtilis, E.coli, S. aureus, P.aeruginosa,* and *K. pneumoniae*, it can confirm that the terpenoids, saponins, tanin, alkaloids, and flavonoids that isolated from *A. concinna* with the chloroform, benzene, methanol, and aqueous extraction condition [36]. The other study of bark extract is effective against *S.typhi, P.nirabilis, S.aureus, Yersinia, S.epidermis, K.pnemonia, P. aerogenosa, E.coli,* and *B.subtilis* under methanol, ethanol, and chloroform extraction condition. These extraction can extracted active compounds included Phenol, Tannin, fat and fixed oil, Flavanoids, Saponin, and quinine [29]. It means that all these compounds have antibacterial activity against most bacteria used in this experiment.

P. niruri, the results of this experiment indicated that the *P. niruri* 95% ethanol and chloroform crude extracted showed antibacterial activity against five bacteria that used in this experiment excepted for *S. enterica* Enteritidis (human) and *E. coli* ATCC25822, respectively. For other three extraction conditions had no present the antibacterial activity of *E. acicularis* extracted. The highest inhibition is on *L. monocytogenes* 10403S which diameter of clear zone is 8.25±1.50 mm for 95%Ethanol extraction condition and on *S. enterica* Typhimurium U302 (DT1046) 10.25±1.50 mm for Chloroform extraction condition, respectively. In addition for the MIC and MBC determination, the crude extract can inhibit *E. coli* ATCC 25822 and *S. enterica* Typhimurium U302 (DT1046) were inhibit and kill in low concentration which is 64 µl/ml for 95%Ethanol extraction condition. For the chloroform extraction condition, the crude extract can inhibit and kill *S. enterica* Enteritidis (human), and *S. enterica* 4,5,12: i-(human) US clone in minimum concentration which is 32 µl/ml. *P. niruri* contained flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, have been identified from various parts have effect to against the Hepatitis B and other viral infections. [30]. The other study report that this plant extract is effective against *Staphylococcus*, *Micrococcus*, and *Pasteurella* bacteria under methanol, DCM with methanol (1:1), and aqueous extract. These are some active compounds that can extractf rom this plant,

Alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, ellagitannins, gallocatechins, geraniin, hypophyllanthin, lignans, lintetralins, and lupeols [31]. The *P. niruni* extracted have antibacterial activity against *Candida albicans, B. pumilus, Micrococcus luteus, K. pneumonia, S. aureus, B. subtilis,* and *E. coli* under mathanol extraction condition. These are the active compounds that can extract from this plant flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins [32].

T. cordifolia, the results of this experiment indicated that the *T. cordifolia* extracted for chloroform crude extracted showed antibacterial activity against all six bacteria that used in this experiment. While *T. cordifolia* 95% Ethanol extracted showed antibacterial activity against five bacteria except for *S. enterica* Enteritidis (human). For other three extraction conditions had no present the antibacterial activity of *T. cordifolia* extracted. The highest inhibition is on *S. enterica Typhimurium U302 (DT1046)* which diameter of clear zone is 9.00±1.41 mm for 95%Ethanol extraction condition and 10.50±2.08 mm for Chloroform extraction condition, respectively. In addition for the MIC and MBC determination, the crude extract can inhibit *E. coli* ATCC25822, *S. enterica* Typhimurium U302 (DT1046), and *S. enterica* 4,5,12: i-(human) US clone were inhibit by 128 µl/ml and kill by 64 µl/ml for 95%Ethanol extraction condition. For the chloroform extraction condition, the crude extract can inhibit and kill *E. coli* ATCC25822 in minimum concentration which is 32 µl/ml.
T. cordifolia contained "alkaloids, glycosides, diterpenoid lactones, sesquiterpenoids and steroids" have been isolated from the *T. cordifolia* [33]. The other study of this plant of ether extract of stem (aerial part) is effective against *Mycobacterium tuberculosis* and aqueous extract is effective against *E. coli* and *S. aureus* with active compounds included alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds, and polysaccharides [34]. In the same of extracted part of this plant under aqueous, ethanol, methanol, and acetone extraction condition have antibacterial activity against *E. coli, S. aureus, K. pneumoniae, P. vulgaris, S. typhi, S. flexneri, S. paratyphi, S. typhimurium, P. aeruginosa, E. aerogene,* and *Serratia marcesensesi* with active compounds included β-sitosterol, hydroxy ecdysone, ecdysterone, and giloinsterol [35],[36].

The molecular polarity of the solvents, they are both polar and non-polar solvents that had study in this experiment. The polar solvents are included water for both sterile distilled water and autoclaving at 121°C PSI 15 for 15 minutes extraction conditions, and ethanol. The non-polar solvents are included chloroform and hexane. Nonpolar molecules can classify into two

classes, molecules with no bond dipoles and molecules with symmetrical bond dipoles that molecules with insignificantly differences in atom electronegativity. For polar molecules, various in their extent of polarity. The relative polarity of 95% ethanol, sterile distilled water and autoclaving at 121°C PSI 15 for 15 minutes, chloroform and hexane extraction conditions are arranged in level from non-polar to polar which are hexane, chloroform, ethanol, and water, respectively. For their polarity index ranged from high to low are water (9.0), ethanol (5.2), chloroform (4.1), and hexane (0.0), respectively. The polarity index is the measurement of the relative polarity of solvent and it is useful for identify the suitable mobile phase of the solvents. The polarity index is also increases with polarity. Moreover, the dielectric constants of these solvent used in this experiment are ranged from high to low; water (80), ethanol (24.3), chloroform (4.8), and hexane (1.9), respectively. The dielectric constant is the measure of the ability of the solvents to separate ionic charges and it is related to the polarity of the solvents. The solubility in water of all four solvents are range from high to low; water and ethanol (100%), chloroform (0.815%), and hexane (0.001%), respectively [24], [25]. From their polarity, it means that the different active compounds can extracted with different solvents it is depended on their charges. Moreover, in same solvent but different extraction condition which are sterile distilled water and autoclaving at 121°C PSI 15 for 15 minutes. The temperature and pressure are the factors that effect to extract the active compound from the plants. Mostly, 95% ethanol and chloroform can extracted the active compounds for against the bacteria. In addition, ethanol and chloroform are polar and non-polar, respectively. So that, that compounds that can extracted might be different including for their solubility in water and the compound content.

CONCLUSION

For the 95% Ethanol and Chloroform extraction condition give the highest antibacterial activity of all herbs against all bacteria and the range of antibacterial activity is between 8.5 mm to 11.5 mm. The best antibacterial activity is 11.50±1.29 mm of *C. nutans* extracted under Chloroform extraction condition on *S. enterica* Typhimurium U302 (DT1046).

For the range of MIC and MBC is between 32µl/ml to 256 µl/ml and the best MIC and MBC is 32 µl/ml of *A. paniculata* extracted against *S. enterica* 4,5,12: i-(human) US clone, *B. cereus, and L. monocytogenes* 10403S

The molecular polarity of the solvents, they are both polar (water for both sterile distilled water and autoclaving at 121°C PSI 15 for 15 minutes extraction conditions) and non-polar (chloroform and hexane) solvents that had study in this experiment.

These results showed the promising of antibacterial activity of three Thai local herbs which are stepping stone for further application like food industry, pharmaceutical industry, and cosmetic industry.

REFERENCES

1. U.S. Food and Drug Administration (2012). Bad Bug book (Second edition) Foodborne Pathogenic Microorganisms and Natural Toxin Handbook.

2. Foodsafety (2013), *Escherichia coli*, retrieved on October 20, 2013 from http://www.foodsafety.gov/poisoning/causes/bacteriaviruses/ecoli/

3. WebMD (2005-2013), *Escherichia coli* Infection, retrieved on October 20, 2013 from http://www.webmd.com/a-to-z-guides/e-coli-infection-topic-overview

4. Nagaprashanthi.Ch*, Rafi khan.P, Gopi chand.K, Aleemuddin MA, Rajiya Begum.G. (2012). In vitro Antimicrobial Activity of *Tinospora cordifolia* and its Phytochemical screening. International Journal of PharmTech Research. Vol.4, No.3, pp 1004-1008.

5. Pannangpetch, P., Laupattarakasem, P., Kukongviriyapan, V., Kukongviriyapan, U., Kongyingyoes, B. and Aromdee, C. (2007). Antioxidant activity and protective effect against oxidative hemolysis of *Clinacanthus nutans* (Burm.f) Lindau. Songklanakarin J. Sci. Technol., March 2007, 29(Suppl. 1) : 1-9.

6. Santi Sakdarat, Aussavashai Shuyprom, Thaweephol Dechatiwongse Na Ayudhya, Peter G. Waterman, Gloria Karagianis (2009). Chemical composition investigation of the *Clinacanthus nutans* Lindau leaves. *Thai Journal of Phytopharmacy Vol. 13(2).*

7. Ho Shing Yang, Tiew Wah Peng, Priya Madhavan, Mohamed Saleem Abdul, Shukkoor, Gabriel Akyirem Akowuah (2013). Phytochemical Analysis and Antibacterial activity of Methanolic extract of *Clinacanthus nutans* Leaf. Int. J. Drug Dev. & Res., July-September 2013, 5 (3): 349-355.

8. Centers for Disease Control and Prevention (2010), Multistate Outbreak of *Salmonella* Typhimurium Infections Linked to Peanut Butter, 2008–2009 (Final Update), retrieved on October 20, 2013 from http://www.cdc.gov/salmonella/typhimurium/update.html#outbreakinvestigation

9. Centers for Disease Control and Prevention (2011), Multistate Outbreak of Human *Salmonella* Enteritidis Infections Linked to Alfalfa Sprouts and Spicy Sprouts, retrieved on October 21, 2013 from http://www.cdc.gov/salmonella/sprouts-enteritidis0611/070611/index.html

10. Centers for Disease Control and Prevention (2012), Multistate Outbreak of Human *Salmonella* Infantis Infections Linked to Dry Dog Food (Final Update), retrieved on October 21, 2013 from http://www.cdc.gov/salmonella/dog-food-05-12/index.html

11. Asst. Prof. Dr. Wunwisa Krasaekoopt (2008), Determination of *Salmonella* sp., Food Microbiology Practical Manual 1st edition, page 72-79, Faculty of Biotechnology, ISBN 974-615-269-6, Assumption University, Thailand

12. Centers for Disease Control and Prevention (2013), Multistate Outbreak of Human *Salmonella* Typhimurium Infections Linked to Live Poultry in Backyard Flocks, retrieved on October 21, 2013 from http://www.cdc.gov/salmonella/typhimurium-live-poultry-04-13/index.html

13. Centers for Disease Control and Prevention (2012), Multistate Outbreak of Listeriosis Linked to Imported Frescolina Marte Brand Ricotta Salata Cheese (Final Update), retrieved on October 21, 2013 from http://www.cdc.gov/listeria/outbreaks/cheese-09-12/

14. Infection Control Today (ICT) (2013), FDA Investigates Multi-State Outbreak of Listeria Monocytogenes Linked to Cheese, retrieved on October 21, 2013 from http://www.infectioncontroltoday.com/news/2013/07/fda-investigates-multistate-outbreak-of-listeria-monocytogenes-linked-to-cheese.aspx

15. Asst. Prof. Dr. Wunwisa Krasaekoopt (2008), Determination of *Listeria monocytogenes*, Food Microbiology Practical Manual 1st edition, page 36-103, Faculty of Biotechnology, ISBN 974-615-269-6, Assumption University, Thailand

16. Katelijne Dierick, Els Van Coillie, Izabela Swiecicka, Geert Meyfroidt, Hugo Devlieger, Agnes Meulemans, Guy Hoedemaekers, Ludo Fourie, Marc Heyndrickx, and Jacques Mahillon (2005), Outbreak of *Bacillus cereus*, Fatal Family Outbreak of *Bacillus cereus*-Associated Food Poisoning, Journal of Clinical Microbiology, Vol. 43, No. 8, p. 4277-9.

17. Juntima Soponsakulkaew and Jaruwan Ngamkam (2010). Antioxidant activity of Purprish Red Herbs. Special project for Bachelor degree of Science in Pharmacy. Faculty of Pharmacy. Mahidol University.

18. Karla M. Rodríguez Tirado (2011). Phytochemical screening of medicinal plants from several genera with antidiabetic activities. Universidad de Puerto Rico.

19. Claribel Luciano-Montalvo, Isabelle Boulogne and Jannette Gavillán-Suárez (2013). A screening for antimicrobial activities of Caribbean herbal remedies. BMC Complementary and Alternative Medicine; 13:126.

20. MD, PhD, Shahid Akbar (2011). Andrographis paniculata: A Review of Pharmacological Activities and Clinical Effects. Qassim University, Saudi Arabia. Alternative Medicine Review; 16 (1) page 66-77.

21. Ghosh Kumar Benoy, Datta Kumar Animesh, Mandal Aninda, Dubery Kumari Priyanka and Halder Sandip (2012). An overview on *Androgaphis paniculata* (Burm.F.) Nees. University of Kalyani. India.

22. Naiyana Tipakorn (2002). Effects of *Andrographis Paniculata* (Burm. F.) Nees on Performance, Mortality and Coccidiosis in Broiler Chickens. Instyte of Animal Physiology and Animal Nutrition. Doctoral Dissertation for Doctor Degree of Agricultural Sciences. Faculty of Agricultural Sciences. Georg-August-University, Germany.

23. B.P. Koch, J. Rullkötter and R.J. Lara (2003). Evaluation of triterpenols and sterols as organic matter biomarkers in a mangrove ecosystem in northern Brazil. *Wetlands Ecology and Management* ; 11: 257–263.

24. Paul Sadek (2002). Solvent Miscibility and Viscosity Chart adapted. The HPLC Solvent Guide Wiley-Interscience.

25. Chapter5: Classification of Organic Compounds by Solubility. Lecture note. retrieved on November 27th, 2013 from http://opencourseware.kfupm.edu.sa/colleges/cs/chem/chem303/files%5C3-Lecture_Notes_CHEM-303_(Chapter_5).pdf> (November, 2013)

26. Mr. Nateepat P. (2010). The study of antibacterial effect in *chrysanthemum indicum, centella asiatica* and *andrographis paniculata* on *Bacillus cereus* and *Listeria monocytogenes* (food-borne pathogenic bacteria) under normal and osmotic stress condition. Special project for Bachelor degree. Assumption University. Thailand.

27. Ms. Treuktongjai S. (2012). Antibacterial activity of Thai red curry paste's ingredients between Using Kang-Pa model and Kang-Kati model on *Salmonella* sp. and *Listeria monocytogenes* 10403S. Special project for Bachelor degree. Assumption University. Thailand.

28. Sandip S. Todkar, V. V. Chavan and A. S. Kulkarni (2010). Screeninh os Secondary Metaboltes and Antibacterial Activity of *Acacia concinna*. Research Journal of Microbiology; 5 (10): 974-979.

29. Xavier Vergeese raja and Rajeshwari Sivaraj (2012). Antibacterial activity of Bark extract of *Acacia concinna* (L). Karpagam University. India.

30. Paithankar V.V., Raut K. S., Charde R. M., and Vyas J. V. (2011). *Phyllanthus Niruri:* A magic Herb. Research in Pharmacy; 1(4): 1-9.

31. Leslie Taylor (2003). Technical Data Report for Chanca Piedra "Stone Breaker" (*Phyllanthus niruri*), *Herbal Secrets of the Rainforest*; 2nd edition.

32. Anthony D Njoroge, B Anyango, SF Dossaji (2012). Screening of *Phyllanthus* Species for Antimicrobial Properties. Chemical Sciences Journal: CSJ-56

33. L. N. Sankhala, R. K. Saini, and B. S. Saini (2012). A review on chemical and biological properties of *Tinospora cordifolia*, Int. J. Med. Arom. Plants; Vol 2, No. 2, 340-344.

34. S. S. Singh, S. C. Pandey, S. Srivastava, V.S. Gupta, and B. Patro, and A.C. Ghosh (2003). Chemistry and Medicinal Properties of *Tinospora cordifolia* (Guduchi), Indian Journal of Pharmacology; 35: 83-91.

35. Soham Saha, Shyamasree Ghosh (2013). *Tinospora cordifolia*: One plant, many roles, Ancient Science of Life; 31: 151-159.

36. Tambekar, D.H., B. S. Khante, B.R.Chandak, A.S.TItare, S.S.Boralkar, and S.N.Aghadte (2009). Screening of Antibacterial potentials of some medicinal plants from melighat forest in India, *Afr. J. Traditional*. Complementary and Alternative Medicines; 6 (3): 228-232

37. Sriwanthana, B., Treesangsri, W., Boriboontrakul, B., Niumsakul, S., and Chavalittumrong, P. (2007), *In vitro* effects of Thai medicinal plants on human lymphocyte activity Songklanakarin J. Sci. Technol., 29(Suppl. 1) : 17-28

38. Sujittapron Sittiso, Tipaya Ekalaksananan, Chamsai Pientong, Santi Sakdarat, Nicha Charoensri, Bunkerd Kongyingyoes (2010). Effects of Compounds from *Clinacanthus Nutans* on Dengue Virus Type 2 Infection, Srinagarind Med J, 25 (Suppl).

39. Aquarium and Pond Plants of the World (2007). ***Eleocharis*** R. Br., *Eleocharis acicularis,* retrieved on November 27th, 2013 from http://idtools.org/id/aquariumplants/Aquarium_&_Pond_Plants_of_the_World/key/Aquarium_&_Pond_Plants/Media/Html/Fact_sheets/eleocharis.html.

40. Raimundo Fernandes de Araújo Júnior, Luiz Alberto Lira Soares, Cínthia Raquel da Costa Porto, Ranniere Gurgel Furtado de Aquino, Hugo Gonçalo Guedes, Pedro Ros Petrovick, Tatiane Pereira de Souza, Aurigena Antunes de Araújo, Gerlane Coelho Bernardo Guerra (2010). Growth inhibitory effects of Phyllanthus niruri extracts in combination with cisplatin on cancer cell lines. *World J Gastroenterol* 21; 18(31): 4162-4168.

41. Aksoy, Murat Yildirim, Birgül Kacmaz, Teoman Zafer Apan, J. Sedef Gocmen (2005), Verotoxin Production in Strains of Escherichia coli Isolated from Cattle and Sheep, and Their Resistance to Antibiotics, Turk. J. Vet. Anim. Sci. 2007; 31(4): 225-231

APPENDIXES

1. *E. coli* ATCC25822

Table 13: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *E. coli* ATCC25822

Herb	*Penicillin-G		*95% Ethanol		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1	2	1	2		
T. spathacea	45	44	10	9	9	8	7	7	7.75	0.96
A. paniculata	46	45	9	8	7	7	7	8	7.25	0.50
C. nutans	44	43	9	9	8	9	9	8	8.5	0.58
E. acicularis	45	44	9	8	7	9	9	7	8	1.15
A. concinna	46	45	8	8	7	7	8	7	7.25	0.50
P. niruri	44	44	9	9	8	9	8	8	8.25	0.50
T. cordifolia	46	45	10	9	9	7	7	8	7.75	0.96
			Mean	8.83						
			SD	0.75						

Herb	*Penicillin-G		*Sterile Distilled water		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	45	44	-	-	-	-	-	-	-	-
A. paniculata	46	45	-	-	-	-	-	-	-	-
C. nutans	44	45	-	-	-	-	-	-	-	-
E. acicularis	43	45	-	-	-	-	-	-	-	-
A. concinna	45	45	-	-	-	-	-	-	-	-
P. niruri	46	44	-	-	-	-	-	-	-	-
T. cordifolia	45	44	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Herb	*Penicillin-G		Autoclaving at 121°C 15 PSI for 15 minutes		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	43	44	-	-	-	-	-	-	-	-
A. paniculata	45	46	-	-	-	-	-	-	-	-
C. nutans	45	45	-	-	-	-	-	-	-	-
E. acicularis	47	47	-	-	-	-	-	-	-	-
A. concinna	46	45	-	-	-	-	-	-	-	-
P. niruri	45	46	-	-	-	-	-	-	-	-
T. cordifolia	47	48	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Table 13: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *E. coli* ATCC25822 (Cont.)

Herb	*Penicillin-G		*Hexane		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	46	45	-	-	-	-	-	-	-	-
A. paniculata	44	44	-	-	-	-	-	-	-	-
C. nutans	45	45	-	-	-	-	-	-	-	-
E. acicularis	46	44	-	-	-	-	-	-	-	-
A. concinna	46	45	-	-	-	-	-	-	-	-
P. niruri	46	47	-	-	-	-	-	-	-	-
T. cordifolia	48	45	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						
Herb	***Penicillin-G**		***Chloroform**		**Crude extract**				**Mean**	**SD**
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	45	44	10	8	-	-	-	-	-	-
A. paniculata	46	47	8	9	-	-	-	-	-	-
C. nutans	46	46	9	9	9	8	8	8	8.25	0.5
E. acicularis	44	44	10	9	-	-	-	-	-	-
A. concinna	43	43	9	9	7	7	7	8	7.25	0.5
P. niruri	46	46	8	9	-	-	-	-	-	-
T. cordifolia	45	44	8	9	7	7	8	7	7.3	0.5
	Mean	45.09	**Mean**	8.9						
	SD	1.15	**SD**	0.66						

Note: "*" positive control (solvents used and Penicillin-G)

"-" no antibacterial activity was presented

- Autoclaving at 121°C 15 PSI for 15 minutes used sterile distilled water as control

2. *S. enterica* Typhimurium U302 (DT1046)

Table 14: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *S. enterica* Typhimurium U302 (DT1046)

Herb	*Penicillin-G		*95% Ethanol		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1	2	1	2		
T. spathacea	19	18	11	9	9	9	10	9	9.25	0.5
A. paniculata	17	17	9	10	9	9	7	8	8.25	0.96
C. nutans	20	19	10	11	7	8	7	8	7.5	0.58
E. acicularis	19	18	12	11	12	11	9	7	9.75	2.22
A. concinna	19	20	10	10	7	10	11	7	8.75	2.06
P. niruri	20	18	9	10	8	7	8	7	7.5	0.58
T. cordifolia	22	20	11	10	10	9	10	7	9	1.41
			Mean	10.21						
			SD	0.89						

Herb	*Penicillin-G		*Sterile Distilled water		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	17	18	-	-	-	-	-	-	-	-
A. paniculata	18	18	-	-	-	-	-	-	-	-
C. nutans	19	18	-	-	-	-	-	-	-	-
E. acicularis	20	17	-	-	-	-	-	-	-	-
A. concinna	18	19	-	-	-	-	-	-	-	-
P. niruri	20	19	9	8	8	7	8	8	7.75	0.50
T. cordifolia	17	18	8	7	8	9	7	7	7.75	0.96
			Mean	7.75						
			SD	0.71						

Herb	*Penicillin-G		Autoclaving at 121°C 15 PSI for 15 minutes		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	17	17	-	-	-	-	-	-	-	-
A. paniculata	18	16	-	-	-	-	-	-	-	-
C. nutans	17	18	-	-	-	-	-	-	-	-
E. acicularis	16	17	-	-	-	-	-	-	-	-
A. concinna	19	19	-	-	-	-	-	-	-	-
P. niruri	21	18	-	-	-	-	-	-	-	-
T. cordifolia	20	21	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Table 14: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *S. enterica* Typhimurium U302 (DT1046) (Cont.)

Herb	*Penicillin-G		*Hexane		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	17	17	-	-	-	-	-	-	-	-
A. paniculata	16	15	-	-	-	-	-	-	-	-
C. nutans	16	16	-	-	-	-	-	-	-	-
E. acicularis	15	17	-	-	-	-	-	-	-	-
A. concinna	19	19	-	-	-	-	-	-	-	-
P. niruri	17	18	-	-	-	-	-	-	-	-
T. cordifolia	16	16	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						
Herb	***Penicillin-G**		***Chloroform**		**Crude extract**				**Mean**	**SD**
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	18	18	12	10	11	12	10	9	10.5	1.29
A. paniculata	19	17	11	10	11	7	10	10	9.5	1.73
C. nutans	18	18	12	10	10	12	13	11	11.5	1.29
E. acicularis	17	19	12	10	8	12	10	12	10.5	1.91
A. concinna	18	18	12	12	9	10	10	11	10	0.82
P. niruri	17	19	10	12	9	11	12	9	10.25	1.5
T. cordifolia	16	17	9	10	11	10	8	13	10.5	2.08
	Mean	17.99	**Mean**	10.86						
	SD	1.44	**SD**	1.1						

Note: "*" positive control (solvents used and Penicillin-G)

"-" no antibacterial activity was presented

- Autoclaving at 121°C 15 PSI for 15 minutes used sterile distilled water as control

3. *S. enterica* Enteritidis (human)

Table 15: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *S. enterica* Enteritidis (human)

Herb	*Penicillin-G		*95%Ethanol		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1	2	1	2		
T. spathacea	38	40	25	21	10	8	8	9	8.75	0.96
A. paniculata	39	41	21	22	7	7	8	7	7.25	0.50
C. nutans	38	40	22	20	-	-	-	-	-	-
E. acicularis	37	37	20	19	-	-	-	-	-	-
A. concinna	40	40	20	21	-	-	-	-	-	-
P. niruri	39	40	21	21	-	-	-	-	-	-
T. cordifolia	37	38	20	20	-	-	-	-	-	-
			Mean	20.93						
			SD	1.44						

Herb	*Penicillin-G		*Sterile Distilled water		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	37	35	-	-	-	-	-	-	-	-
A. paniculata	37	36	-	-	-	-	-	-	-	-
C. nutans	36	37	-	-	-	-	-	-	-	-
E. acicularis	38	40	-	-	-	-	-	-	-	-
A. concinna	34	36	-	-	-	-	-	-	-	-
P. niruri	36	35	-	-	-	-	-	-	-	-
T. cordifolia	39	37	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Herb	*Penicillin-G		Autoclaving at 121°C 15 PSI for 15 minutes		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	35	35	-	-	-	-	-	-	-	-
A. paniculata	35	36	-	-	-	-	-	-	-	-
C. nutans	37	36	-	-	-	-	-	-	-	-
E. acicularis	37	36	-	-	-	-	-	-	-	-
A. concinna	35	35	-	-	-	-	-	-	-	-
P. niruri	37	38	-	-	-	-	-	-	-	-
T. cordifolia	39	38	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Table 15: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *S. enterica* Enteritidis (human) (Cont.)

Herb	*Penicillin-G		*Hexane		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	37	38	-	-	-	-	-	-	-	-
A. paniculata	38	39	-	-	-	-	-	-	-	-
C. nutans	39	37	-	-	-	-	-	-	-	-
E. acicularis	37	37	-	-	-	-	-	-	-	-
A. concinna	38	37	-	-	-	-	-	-	-	-
P. niruri	36	37	-	-	-	-	-	-	-	-
T. cordifolia	38	39	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						
Herb	***Penicillin-G**		***Chloroform**		**Crude extract**				**Mean**	**SD**
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	38	37	11	12	9	7	8	11	8.75	1.71
A. paniculata	37	38	13	11	10	9	8	7	8.5	1.29
C. nutans	37	38	10	12	11	9	11	10	10.25	0.96
E. acicularis	39	38	11	10	8	10	12	10	10	1.63
A. concinna	38	40	13	10	8	8	9	8	8.25	0.5
P. niruri	37	40	12	12	7	9	11	10	9.25	1.71
T. cordifolia	38	39	10	11	12	9	7	9	9.25	2.06
	Mean	37.49	**Mean**	11.29						
	SD	1.52	**SD**	1.07						

Note: "*" positive control (solvents used and Penicillin-G)

"-" no antibacterial activity was presented

- Autoclaving at 121°C 15 PSI for 15 minutes used sterile distilled water as control

4. *S. enterica* 4,5,12: i-(human) US clone

Table 16: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *S. enterica* 4,5,12: i-(human) US clone

Herb	*Penicillin-G		*95% Ethanol		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1	2	1	2		
T. spathacea	25	25	8	8	8	11	10	8	9.25	1.5
A. paniculata	24	26	9	8	9	11	8	9	9.25	1.26
C. nutans	26	26	9	9	7	9	9	8	8.25	0.96
E. acicularis	27	25	9	8	7	7	8	9	7.75	0.96
A. concinna	27	26	7	8	9	11	10	12	10.5	1.29
P. niruri	25	25	8	8	10	8	8	9	8.75	0.96
T. cordifolia	24	25	9	8	8	8	9	8	8.25	0.5
			Mean	8.29						
			SD	0.61						

Herb	*Penicillin-G		*Sterile Distilled water		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	27	25	-	-	-	-	-	-	-	-
A. paniculata	31	29	-	-	-	-	-	-	-	-
C. nutans	27	25	-	-	-	-	-	-	-	-
E. acicularis	26	25	-	-	-	-	-	-	-	-
A. concinna	27	25	-	-	-	-	-	-	-	-
P. niruri	27	25	-	-	-	-	-	-	-	-
T. cordifolia	26	25	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Herb	*Penicillin-G		Autoclaving at 121°C 15 PSI for 15 minutes		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	25	26	-	-	-	-	-	-	-	-
A. paniculata	25	25	-	-	-	-	-	-	-	-
C. nutans	27	25	-	-	-	-	-	-	-	-
E. acicularis	27	26	-	-	-	-	-	-	-	-
A. concinna	27	25	-	-	-	-	-	-	-	-
P. niruri	24	25	-	-	-	-	-	-	-	-
T. cordifolia	25	26	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Table 16: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *S. enterica* 4,5,12: i-(human) US clone (Cont.)

Herb	*Penicillin-G		*Hexane		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	26	25	-	-	-	-	-	-	-	-
A. paniculata	25	27	-	-	-	-	-	-	-	-
C. nutans	24	25	-	-	-	-	-	-	-	-
E. acicularis	24	27	-	-	-	-	-	-	-	-
A. concinna	26	24	-	-	-	-	-	-	-	-
P. niruri	25	25	-	-	-	-	-	-	-	-
T. cordifolia	27	26	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						
Herb	***Penicillin-G**		***Chloroform**		**Crude extract**				**Mean**	**SD**
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	24	25	11	9	9	10	10	8	9.25	0.96
A. paniculata	26	25	8	11	12	10	11	10	10.75	0.96
C. nutans	25	24	10	8	10	13	10	11	11	1.41
E. acicularis	24	26	8	8	8	9	9	8	8.5	0.58
A. concinna	27	26	9	10	10	10	9	8	9.25	0.96
P. niruri	25	24	11	11	11	10	9	9	9.75	0.96
T. cordifolia	26	26	10	9	8	11	9	9	9.25	1.26
	Mean	25.63	**Mean**	9.5						
	SD	1.22	**SD**	1.22						

Note: "*" positive control (solvents used and Penicillin-G)

"-" no antibacterial activity was presented

- Autoclaving at 121°C 15 PSI for 15 minutes used sterile distilled water as control

5. *B. cereus*

Table 17: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *B. cereus*

Herb	*Penicillin-G		*95%Ethanol		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1	2	1	2		
T. spathacea	21	19	7	8	9	7	8	8	8	0.82
A. paniculata	18	19	8	8	9	8	8	7	8	0.82
C. nutans	20	19	9	8	7	7	7	8	7.25	0.5
E. acicularis	20	20	9	8	9	9	8	8	8.5	0.58
A. concinna	20	21	7	7	7	7	8	7	7.25	0.5
P. niruri	21	20	10	9	7	7	8	8	7.5	0.58
T. cordifolia	20	19	8	9	7	7	7	8	7.25	0.5
			Mean	8.21						
			SD	0.89						

Herb	*Penicillin-G		*Sterile Distilled water		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	21	19	-	-	-	-	-	-	-	-
A. paniculata	18	19	-	-	-	-	-	-	-	-
C. nutans	20	19	-	-	-	-	-	-	-	-
E. acicularis	20	13	-	-	-	-	-	-	-	-
A. concinna	21	21	-	-	-	-	-	-	-	-
P. niruri	21	20	-	-	-	-	-	-	-	-
T. cordifolia	20	19	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Herb	*Penicillin-G		Autoclaving at 121°C 15 PSI for 15 minutes		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	21	20	-	-	-	-	-	-	-	-
A. paniculata	20	20	-	-	-	-	-	-	-	-
C. nutans	19	21	-	-	-	-	-	-	-	-
E. acicularis	18	19	-	-	-	-	-	-	-	-
A. concinna	20	19	-	-	-	-	-	-	-	-
P. niruri	19	21	-	-	-	-	-	-	-	-
T. cordifolia	21	20	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Table 17: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *B. cereus* (Cont.)

Herb	*Penicillin-G		*Hexane		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	20	21	-	-	-	-	-	-	-	-
A. paniculata	19	19	-	-	-	-	-	-	-	-
C. nutans	19	18	-	-	-	-	-	-	-	-
E. acicularis	20	18	-	-	-	-	-	-	-	-
A. concinna	19	21	-	-	-	-	-	-	-	-
P. niruri	20	21	-	-	-	-	-	-	-	-
T. cordifolia	19	19	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						

Herb	*Penicillin-G		*Chloroform		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	18	18	8	8	8	7	8	9	8	0.82
A. paniculata	19	19	8	9	8	8	9	8	8.25	0.5
C. nutans	20	21	8	8	8	9	9	9	8.75	0.5
E. acicularis	20	20	9	8	7	9	8	8	8	0.82
A. concinna	18	19	7	8	8	7	7	8	7.5	0.58
P. niruri	19	21	7	8	8	8	8	9	8.25	0.5
T. cordifolia	20	19	9	9	7	10	9	9	8.75	1.26
	Mean	19.71	**Mean**	8.14						
	SD	1.04	**SD**	0.66						

Note: "*" positive control (solvents used and Penicillin-G)

"-" no antibacterial activity was presented

- Autoclaving at 121°C 15 PSI for 15 minutes used sterile distilled water as control

6. *L. monocytogenes* 10403S

Table 18: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *L. monocytogenes* 10403S

Herb	*Penicillin-G		*95% Ethanol		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1	2	1	2		
T. spathacea	22	18	12	9	6	6	7	6	6.25	0.5
A. paniculata	20	22	8	7	7	6	7	6	6.5	0.58
C. nutans	19	23	8	6	8	7	8	6	7.25	0.96
E. acicularis	20	21	7	10	7	9	9	9	8.5	1
A. concinna	20	19	10	9	7	8	8	6	7.25	0.96
P. niruri	22	20	9	9	9	7	10	7	8.25	1.5
T. cordifolia	23	21	9	8	7	8	7	6	7	0.82
			Mean	8.6						
			SD	1.5						

Herb	*Penicillin-G		*Sterile Distilled water		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	19	18	6	8	-	-	-	-	-	-
A. paniculata	19	18	6	6	-	-	-	-	-	-
C. nutans	19	23	7	8	-	-	-	-	-	-
E. acicularis	21	20	7	7	-	-	-	-	-	-
A. concinna	18	19	8	8	-	-	-	-	-	-
P. niruri	21	22	7	8	-	-	-	-	-	-
T. cordifolia	21	23	8	7	-	-	-	-	-	-
			Mean	7.21						
			SD	0.8						

Herb	*Penicillin-G		Autoclaving at 121°C 15 PSI for 15 minutes		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	17	17	7	8	-	-	-	-	-	-
A. paniculata	18	19	8	8	-	-	-	-	-	-
C. nutans	20	21	9	7	-	-	-	-	-	-
E. acicularis	18	20	8	8	-	-	-	-	-	-
A. concinna	19	19	6	7	-	-	-	-	-	-
P. niruri	21	23	7	7	-	-	-	-	-	-
T. cordifolia	20	21	6	8	-	-	-	-	-	-
			Mean	7.43						
			SD	0.85						

Table 18: Clear zone (mm) of antibacterial activity of seven herbs extracted under different extraction conditions on *L. monocytogenes* 10403S (Cont.)

Herb	*Penicillin-G		*Hexane		Crude extract				Mean	SD
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	19	20	-	-	-	-	-	-	-	-
A. paniculata	18	18	-	-	-	-	-	-	-	-
C. nutans	19	17	-	-	-	-	-	-	-	-
E. acicularis	20	21	-	-	-	-	-	-	-	-
A. concinna	21	21	-	-	-	-	-	-	-	-
P. niruri	21	21	-	-	-	-	-	-	-	-
T. cordifolia	20	22	-	-	-	-	-	-	-	-
			Mean	-						
			SD	-						
Herb	***Penicillin-G**		***Chloroform**		**Crude extract**				**Mean**	**SD**
	1st trial	2nd trial	1st trial	2nd trial	1st trial		2nd trial			
					1st plate	2nd plate	1st plate	2nd plate		
T. spathacea	18	17	7	8	9	8	10	8	8.75	0.96
A. paniculata	19	19	8	10	10	7	10	6	8.25	2.06
C. nutans	20	21	8	8	10	7	10	10	9.25	1.5
E. acicularis	22	17	8	9	10	9	8	10	9.25	0.96
A. concinna	18	18	9	10	8	10	9	10	9.25	0.96
P. niruri	19	20	7	9	9	9	10	12	10	1.41
T. cordifolia	21	18	9	8	10	8	9	8	8.75	0.96
	Mean	19.83	**Mean**	8.43						
	SD	1.65	**SD**	0.94						

Note: "*" positive control (solvents used and Penicillin-G)

"-" no antibacterial activity was presented

- Autoclaving at 121°C 15 PSI for 15 minutes used sterile distilled water as control