

RESULT OF FEW TYPES OF TRADITIONAL HERBAL EXTRACTS ON BACTERIA INHIBITION

Jiraporn¹ Ruankaew¹ Dena¹ Michcal¹

¹Department of Science Service, Bureau of Community Technology, Bangkok University, Chiang Mai

Article Accepted on: August: 06/2020.

Article published on: August: 10/2020.

Abstract

Few types of Thai traditional herbs were extracted using water, methanol and ethanol as solvents. Inhibition of 4 strains of pathogenic bacteria were studied, namely *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC27736, *Staphylococcus aureus* ATCC6538, *Staphylococcus epidermidis* ATCC12228 by Agar well diffusion method. Samples against 1 bacterial strain. The results showed that methanol extract showed the best inhibition of *E. coli* and *S. epidermidis*. The mean inhibitory activity diameter was 20.46 mm and 35.23 mm, respectively. Water-based peppermint extract and methanol extract showed the best inhibitory activity against *K. pneumoniae* and *S. aureus*. The mean diameter of inhibitory activity was 19.15 mm and 24.77 mm, respectively, when tested for the lowest concentration of the extracts capable of inhibiting bacteria by the microdilution assay found that the Minimum Inhibitory Concentration (MIC) of methanol extract with methanol in inhibiting *E. coli* and *S. epidermidis* was 7.81 mg / ml and 62.50 mg / ml, respectively. Water and methanol extract with methanol to inhibit *K. pneumoniae* and *S. aureus* had the MIC value of 15.62 mg / ml and the standard MIC value. Chloramphenicol The inhibitors of *E. coli*, *K. pneumoniae*, *S. epidermidis* and *S. aureus* were 15, 7, 31 and 7 µg / ml, respectively.

KEYWORDS: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*

1. INTRODUCTION

At present there are microorganisms that cause disease. Many in soil, water, air, bacteria that is a major cause of disease that is often found contaminated in the bathroom and in various places: 1) *Escherichia coli* is endemic. In the digestive system Most of them are non-pathogenic but have been found to cause diarrhea, urinary tract infections 1,2 Can be contacted through food or fresh fruit and vegetables.

2) *Klebsiella pneumoniae* is found in the environment, including water, soil, plants and in living organisms' mucous membranes. Gastrointestinal tract, disinfectant, mantle, and human 3 from the airway or lungs. This is the cause of pneumonia, pneumonia, traumatic urinary tract infection in the bloodstream, and meninges that are Gram negative pathogens associated with Infection in the hospital in the respiratory system. Most bottom.

3) *Staphylococcus aureus* is an important pathogen. It is the cause of infection of the skin, wounds and tissues. And food poisoning It is also an opportunistic infection in patients with Weak body Cause pneumonia that causes the mortality rate was as high as 50% ^{6,7}.

4) *Staphylococcus epidermidis* causes purulent inflammatory acne. Found in general skin and some mucous membranes such as nose, ears, mouth and urethra ^{7,8}. Due to the



spread of pathogens in various sources, protection against pathogens should be introduced into the body. One good way to prevent pathogens is Washing hands thoroughly using liquid soap products Or detergent that can kill pathogenic microorganisms Which commercially available products contain chemicals that Help sterilize microorganisms In which consumers may be allergic to chemicals if herbal extracts are used as Ingredients in hand sanitizer products will help in Reducing the use of chemicals People with sensitive skin can use it and increase the value of Thai herbs as well. Traditional Thai herbs have many uses, such as food, spices, medicine. Etc., where there are reports of the substance Many important types of oils, volatile oils, antioxidants, antibacterial and antifungal agents. Therefore, 7 types of Thai traditional herbs were selected to be studied as follows.

1) Parsley (*Eryngium foetidum* L.) in the family Apiaceae (Umbelliferae), an important colorant, acetophenon, aldehyde, benzaldehyde, etc., the root promotes appetite.

2) Cha Plu (*Piper sarmentosum*.

Roxb.) Is in the family Piperaceae, contains neolignan, sarmentosine, etc. Research reports have shown that the extraction of rosin leaves with petroleum ether contains hydrocinnamic acid and β -sitosterol¹⁰. And the extract contains pellitorine¹¹. The leaves make the phlegm periodic and dry to nourish the elements, relieve indigestion, colic, and have antibacterial activity. Reduce blood sugar, resist the clotting of platelets.

3) Peppermint (*Mentha cordifolia* Opiz.) In the family Lamiaceae contains important substances cadinene, carvone, coumarin, etc. and in mint leaves contains amino acid, namely leucine, methionine, proline, asparagine, etc. Tumor growth Lowering blood pressure, stimulating uterine contractions.

4) Meo (*Sechium edule* Sm.), Belonging to the family Cucurbitaceae, the main substances found are flavonoids, 3 C-glycosyl, 5 o-glycosyl flavones. Atherosclerosis, inflammation, boiled water, leaves and the results are used in the symptoms. Hardened blood vessels High blood pressure and breakdown of kidney stones.

5) Basil (*Ocimum basilicum* L.), belonging to the Lamiaceae family, leaves contain essential oils, methyl chavicol and linalool, expel the wind, relieve bloating, seeds when soaked in water will swell into mucus as a laxative due to increasing the amount of food residue.

6) Holy basil (*Ocimum tenuiflorum* L.) is in the family Lamiaceae contains important substances anethole, benzaldehyde, ascorbic acid, essential oil, eugenol¹³ etc. Help digestion, relieve stomach pain, have antifungal and antibacterial effects. Inhibits the growth of cancer cells ¹⁴ anti-inflammatory effects ¹⁵ Stimulates immunity Lowering cholesterol Reduce blood fat.

7) Pandanus (*Pandanus amaryllifolium*.



Roxb.) In the Pandanaceae family contains important substances benzylacetate, carotenoids, coumarin, geraniol, etc. Used for fever, heat, thirst, fatigue, diuretic, lowering blood pressure. Decreased heart rate and pandan root extract Hypoglycemic activity.

2. METHOD

1. Extraction of herbal substances Wash all 7 types of fresh herbs. Clean, desiccate in the shade and extracted as follows

1.1 Extraction of herbs with water Take fresh herbs and cut into small pieces.

Water in an herb to water ratio equal to 1: 4, boiled at 100 ° C for 10 minutes, filtered with a thin white cloth and then filtered with Whatman® No.4 filter paper. Celsius

1.2 Herbal extraction with methanol and ethanol the fresh herbs are cut into small pieces and dried at 50 ° C using a steam oven, grind the sample thoroughly and mix with Methanol or ethanol with the herb to solvent ratio of 1: 4, fermented at room temperature for 24 hours, filtered with filter paper. Whatman® No.4 Keep the solution part, repeat 2 more times and darken the whole solution. Concentrated by evaporator under vacuum, the viscous substance is stored at -20 ° C temperature.

The extracts from clauses 1.1 and 1.2 are dissolved in 1% dimethyl sulfoxide solution and filtered through a sterile 0.2-micron filter prior to use.

2. Tests for antibacterial activity of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis* and *Staphylococcus aureus* of herbal extracts. 2.1 Each bacterial culture. Species in the nutrient broth medium at a shaking speed of 200 rpm for 12 hours at a temperature of 37 ° C. 2.2 The activity of extracts was investigated by Agar well diffusion method, Agar well diffusion method modified from 17. Rauha et al., 2000. Bacteria cultured in item 2.1; 108 CFU / ml cell count were spread over the nutrient agar medium with sterile cotton swab. The results were measured by measuring the diameter of the inhibition zone. Each experiment was obtained by adding 300 µg / ml of 100 µl of herbal extracts to the incubator at 37 ° C for 24 hours. Sample made Repeat 3 times

2.3 Yes, the minimum inhibitory concentration (MIC) concentration of the extract by the microdilution assay. The MIC value is the value of the extract concentration. Concentrate is lowest to inhibit bacteria, which in the application of the extract as a component. In different products such as liquid soap or gel Take an antibiotic bath Will make known the amount of extract at the very least, that should be added to the product.

Determination of the minimum concentration of a substance Extraction that inhibits bacteria Adapted from the method of Sahin et al., 18 2003 using herbal extracts. The dilution was diluted twice by using Muller Hinton broth (MHB) culture medium in 96 well micro plates to a concentration between 0-2.5x10⁴ µg / ml, volume 50 µl, add bacteria, cell count 108 CFU / ml into a well of 50 µl, incubated at 37 oC for 24 hrs, then put in a 0.06 µg / ml concentration of iodinitrotetrazolium chloride. Observe the results, if there is a red color



indicates that Bacteria are not dead and if they are colorless, they are Extraction can inhibit microorganisms because Dead microbes Repeat each experiment by 3. Time.

2.1. MATERIALS AND CHEMICALS

1. Extraction of herbal substances.

1.1 Herbs used in this experiment 7

The species are celery leaves, licorice leaves and stems.

Mint, chives, miao, basil, basil and pandan leaves from a market in Bangkok.

1.2 Methanol

1.3 Ethanol 1.4 Dimethyl sulfoxide (DMSO)

1.5 Whatman® filter paper

No. 4

1.6 Sterile filter, 0.2 micron.

1.7 Evaporator under vacuum

(Rotary evaporator)

2. Microbial culture 2.1 Microorganisms used in research were Courtesy of Chulintharya Research Center.

Science and Technology of Thailand, namely *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC27736, *Staphylococcus aureus* ATCC6538 and *Staphylococcus epidermidis* ATCC12228 Nutrient broth (NB) culture media 3. Antibacterial activity test. Of herbal extracts

3.1 Nutrient medium

agar (NA)

3.2 Muller media Hinton broth (MHB)

3.3 Iodonitrotetrazolium Chloride (Iodonitrotetrazolium chloride)

3.4 Cork borer

3.5 Chloramphenicol (Chloram- phenicol)

STUDY RESULTS

1. Extraction of herbal substances 7 kinds of local herbs when extracted with water, ethanol and methanol. As shown in Table 1, the yield of basil extract with ethanol was obtained.



The highest% yield is 34.65 percent.

2. Antibacterial activity of herbal extracts.

When extracting 7 types of herbs at a concentration of 300 µg / ml was studied in inhibition of all 4 strains of bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* extracted 6, 12, 14 and 9 samples respectively.

(Table 2) that were able to inhibit each strain of bacteria. Average diameter of activity. The inhibitors ranged from 14.45 to 20.46 mm, 11.69 to 19.15 mm, 16.42 to 35.03 mm, and 15.39 to 24.77 mm, respectively.

(Table 2-3)

Inhibition of *E. coli* contains a number of extracts.

6 samples with anti-*E. coli* activity

Table 1 shows the nature of herbal extracts and the percentage of the extract on dry weight.

Herbs Extracting solvent Percentage of extract

Water parsley, dark brown, viscous 2.24

Dark green ethanol viscous 19.25

Methanol, brown-black, viscous 8.5

Chaplo water, black brown liquid, viscous 7.12

Dark green ethanol viscous 20.56

Methanol dark green viscous 4.19

Viscous brown liquid solution 3.23

Green ethanol viscous 23.00

Methanol green viscous 9.67

Green water, brown, viscous 3.33

Green ethanol concentrates 18.54

Green methanol condensed 20.82

Thyme Brown Juice 4.21

Dark green ethanol viscous 34.65

Methanol dark green viscous 20.00

Sweet Basil, Brown, Viscous 3.21



Dark green ethanol viscous 23.67

Methanol, dark green, viscous 3.49

Toei condensed brown juice 4.11

Green ethanol concentrates 22.12

Green methanol concentrates 28.00

The methanol was extracted with methanol. The center of the inhibitory activity was 20.46 mm tall, with all samples having mean crossings. The center of the inhibitory effect is less than the standard substance. Chloramphenicol at a concentration of 100 µg / ml (26.81 mm) when tested.

The lowest concentrations of miao extract with Methanol against *E. coli* was found to have the same MIC value. 7.81 mg / ml Inhibition of *K. pneumoniae* contains substances Twelve samples were extracted with anti-*K. pneumoniae* activity by peppermint extract. The mean diameter of the highest inhibitory activity was 19.15 ml. Through the central inhibitory activity of 100 µg / ml (15.95 mm) of the standardized Chloramphenicol standard, when tested for the lowest extract concentration Peppermint with water against *K. pneumoniae* was found to have a MIC value of 15.62 mg / ml. Inhibition of *S. epidermidis*. 14 extracts were effective against *S. epidermidis* by methanol extract. The mean diameter of the highest inhibitory activity was 35.23 ml, which is wider than the value. Mean inhibitory activity diameter of 100 µg / ml (22.98 mm) of standardized Chloramphenicol when tested for minimum extract concentration. Peppermint with water against *K. pneumoniae* was found to have a MIC value of 62.50 mg / ml. Inhibition of *S. aureus* contains extracts.

9 samples with anti-*S. aureus* activity by the extract of Cha Plu with methanol were

The mean diameter of the highest inhibitory activity was 24.77 mm with mean diameter. The center of the inhibitory effect is wider than the standard substance. Chloramphenicol at 100 µg / ml (14.56 mm) was tested for the lowest concentration of peppermint extract with water.

Against *K. pneumoniae*, MIC was found. Equals 15.62 milligrams per milliliter

Table 2 Mean, diameter, anti-bacterial activity of herbal extracts.

Kind of Herbs / antibiotics, solvents used in

Mean extraction, diameter, inhibitory activity ± SD (mm)

E. coli *K. pneumoniae* *S. epidermidis* *S. aureus*

Water parsley - 14.04 ± 0.12 16.42 ± 0.38 15.39 ± 0.58

Ethanol - - 22.99 ± 1.77 15.73 ± 1.03



Methanol 18.49 ± 0.04 - 30.71 ± 0.51 19.60 ± 1.21
 Cha Phlu Water - 13.58 ± 1.19 - -
 Ethanol 14.45 ± 0.85 - 26.29 ± 0.17 -
 Methanol 19.65 ± 0.46 - 35.17 ± 1.20 24.77 ± 2.72
 Water Peppermint - 19.15 ± 0.53 24.77 ± 3.72 -
 Ethanol 14.62 ± 0.12 15.15 ± 0.17 21.5 ± 0.96 -
 Methanol - 13.68 ± 0.33 29.29 ± 0.18 17.6 ± 0.11
 Meo water hatch - 13.34 ± 2.71 - -
 Ethanol - - 29.44 ± 0.07 22.40 ± 1.81
 Methanol 20.46 ± 0.15 18.38 ± 0.12 35.23 ± 1.93 21.64 ± 2.30
 Water thyme - 13.56 ± 0.55 - -
 Ethanol - - - -
 Methanol - 16.59 ± 0.12 31.72 ± 0.21 20.67 ± 0.28
 Holy Basil - - - -
 Ethanol - 14.48 ± 0.21 - -
 Methanol 19.76 ± 1.06 - 30.39 ± 2.84 21.23 ± 1.59
 Pandan water - 11.69 ± 0.33 - -
 Ethanol - 13.99 ± 0.09 17.77 ± 0.12 -
 Methanol - - 17.93 ± 0.32 -
 Chloramphenicol 70% Methanol 26.81 15.95 229.8 14.56
 1% DMSO water - - - -

- = No zone

Table 3: Minimum concentrations of herbal extracts against bacterial antibiotic testing

Bacteria

Tested MIC value (mg / ml)

Miao extract

With methanol, peppermint extract



With water extract

With Methanol Chloramphenicol

E. coli 7.81 - - 0.015

K. pneumoniae - 15.62 - 0.007

S. epidermidis 62.50 - - 0.031

S. aureus - - 15.62 0.007

3. REVIEW AND SUMMARIZE THE RESULTS OF THE EXPERIMENT

From the experiment, there were 7 kinds of substances from the extract of herbs, 300 micrograms of concentrated herb extract, and lillithir were tested for the antibacterial effect of 4. Species found that the subspecies and scoring solution had an effect on the antibacterial effectiveness of the methanol. The most reliable treatment was 21 times out of 28 samples while the water extraction was effective in Inhibition of only 9 samples was minimal. Herbal extracts were effective in inhibiting all 4 types of infection, namely parsley extract with methanol and Meo extract with methanol While extract

Holy basil with water was unable to inhibit the 4 microorganisms. The test for the performance of the substances supporting *E. coli* and *S. epidermidis* was found. With methanol, the maximum inhibitory activity diameter was 20.46 mm and 35.23 mm, respectively, and the water extracted with peppermint. *pneumoniae* and *S. aureus* with mean mean diameter of 19.15 ml and 24.77 mm. With 100 µg / ml of chloramphenicol, herbal extracts were found to inhibit *K. pneumoniae*, *S. epidermidis* and *S. aureus*. The mean diameter of the inhibitory activity was wider. There are 3, 10 and 9 types of chloramphenicol, respectively, while no herbal extracts are available. Can inhibit *E. coli* mean line diameter. The middle of the inhibitory effect is wider than chloramphenicol. When testing for the lowest concentrations of extracts capable of *E. coli* and *S. epidermidis*, it was found that methanol scaffold was the most effective at MIC value. Equivalent to 7.81 and 62.50 mg / ml, respectively, and the low concentrations of SCDs, such as *K. pneumoniae* and *S. aureus*, were obtained from peppermint extract with water and alkalis. Aloe Vera with methanol key MIC Equals 15.62 milligrams per milliliter.

From this research, information can be used. Extract that has the effect of inhibiting pathogenic bacteria to use. As an ingredient in cleaning products antibacterial body such as liquid soap or hand soap, shower gel, etc. to reduce the number of pathogenic bacteria, which traditional herbal ingredients are cheap. Easy to grow and large quantity If used to develop Extracts are used as an ingredient in such products to add value to traditional herbs. Thailand is another way too.

4. REFERENCE



Burstein S, Taylor P, EL-Ferally FS, Tumer C. Prostaglandins and cannabis V. Identification of p-vinylphenol as a potent inhibitor of prostaglandin synthesis. *Biochem Pharmacol* 1976; 25 (17): 2003-4.16.

Dey BB, Choudhuri MA. Effect of leaf development stage on changes in essential oil of *Ocimum sanctum* L. *Biochem Physiol Pflanz*. 1983; 178 (5): 331-5.

Faculty of Pharmacy Mahidol University and Center for Genetic Engineering And National Biotechnology Local wood herbs. Volumes 1-4. Edition No. 1-4. People's Company Limited; 1996 - 2000.

Health love club. *Escherichia coli* diarrhea. [Online]. 2011. [Referred to 12 June 2011]. Accessed from: www.thailabonline.com/sec51ecoli.htm.

Jittawan Kubola. Analysis of the Bioactive Substances in Bitter Melon and Its Bioavailability. Master of Science Thesis in Food Technology, Mahasarakham University; 2008.

Mayuree Tantisira, Boonyong Tantisira, Thongchai Suksawet, Piyarat Nimpithakphong, Pornthip Boonchaipa, Rungthip Theplertboon. Initial test for local anesthetic potency of Semi-pure extract from the larva. *Thai Pharmaceutical Substances*. 1999; 23 (1): 41-45.

Nitipong Siriwong, Ekachai Chuakiatirot. Incidence of drug-resistant *Staphylococcus aureus* Antibiotics and metals isolated from hospitals in Chiang Rai, Thailand. [Online]. 33rd Congress on Science and Technology of Thailand; 2011.

Noi Namsa, Kanjan Phromma. Chemical study from Chaplu Piper sarmentosum Roxb. *Prince of Songkla Journal*. 1983; 5 (2): 151-2.

Nonglak Suwannapinit. Bacteria related to disease. Edition 3. Bangkok: Chulalongkorn University Book Center; 2004.

Parichart Phalanisat. Bachelor of Science Thesis in Biology. Chiang Mai University; 2011.

Parichart Phalanisong. Master of Science Thesis in Biology. Chiang Mai University; 2008.

Penchop Phuengwichai, Yuwee Wongkrajang, Orawan Ruangsomboon and Wisuda Suwitthayawat Hypoglycemic effect of Toeihom Root Juice II: Diabetic rats. *Journal of Pharmacy, Mahidol University*. 1990; 17 (2): 29-35.

Pornthip Thitapanich. Effect of extract 70% ethanol from basil leaves on growth of AS-30D interstitial hepatocellular carcinoma cells, Sprague dewley Master of Science Thesis in Food Toxicology and Nutrition. Mahidol University. 2002.15

Prachuap Suksomboon. The study of various substances in the Sarakae leaves (*Mentha viridis*). Degree thesis. Bachelor of Science in Chemistry Teaching Chiang Mai University. 1978.



- Rauha, J, Remes S, Heinonen M, Hopia A, Kahaonen M, Kujala T, et al. Antimicrobial effects of finished plant extracts containing flavonoids and other phenolic compounds. *Int J Food microbiol* 2000; 56 (1): 3-12. 18.
- Sahin F, Karaman I, Gulluce M, Ogutcu H, Sengul M, Adiguzel A, et al. Evaluation of antimicrobial activities of *Satureja hortensis* L.J *Ethnopharmacol* 2003; 87: 61-5.
- Sophon Kongsamran. *Medical bacteria*. Faculty of Medicine Siriraj Hospital Mahidol University. Bangkok; 1981.
- Walapol Khittisanuthuang, Samantha Amphawong, Aramu Sangihathawat Khetcharoen, Yaowalak Phanawetkijkul and Kanchana Kheng Worth. Detection of *Klebsiella pneumoniae* in ICR mouse species. [Online]. National Laboratory Animal Center, Mahidol University. 2012.

