Indonesian Journal of Medical Laboratory Science and Technology

Open Access p-ISSN 2684-6748 | e-ISSN 2656-9825 IJMLST

Combination Test Chinese Leaves Extract (*Leucaena leucocephala folium*) and Aloe Vera Inhibiting Growth *Escherichia coli*

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Received: June 10, 2020
Revised: Juni 29, 2020
Accepted: August 20, 2020

Abstract

Chinese petai (*Leucaena leucocephala folium*) and aloe vera (*Aloe vera* L.) have medicinal properties among the plants. The objective of this study was to determine the number of inhibitory zones produced by the ethanol extract of Chinese petai and aloe vera on the growth of *Escherichia coli*. The research method was In-vitro Experimental Laboratory research design with the Kirby Bauer method. The samples used was Chinese petai and aloe vera with pure strains of *E. coli*. One Way ANOVA was used to compare the differences in inhibition of Chinese petai and aloe vera on the growth of *E. coli*. The results of a combination of Chinese petai and aloe vera extract test showed that there were significant differences in the concentration of 25 g, 50 g, 75 g, and 100 g ($p < 0.05$). The ethanol extract of Chinese petai and aloe vera can inhibit the growth of *E. coli*. From the results of this study found that there was an interaction on the combination of ethanol extract of Chinese petai and aloe vera inhibiting the growth of *E. coli* with the most effective concentration being 100 g/mL. This study can find out the benefits of petai cina and aloe vera also the public will know the benefits and efficacy of Chinese petai and aloe vera leaves in medicine.

Keywords

Leucaena leucocephala folium, *Aloe vera* L., *Escherichia coli*, inhibition, combination

INTRODUCTION

Chinese petai leaf (*Leucaena leucocephala folium*) and aloe vera (*Aloe vera*) are one of the traditional medicines that have the potential to be developed and utilized by the people of Indonesia (1). Chinese petai contains alkaloids, saponins, lectins, tannins, mimosin, leucanin, protein, calcium, phosphorus, iron, fatty acids, fibre, vitamin A and other vitamins. Besides that, Chinese petai leaves also contain various flavonoids that become anti-inflammatory and antioxidants. Lectins-contained to stimulate skin cell growth while the alkaloids function as antimicrobials. Aloe vera beneficial properties, including tannin, amino

acids, anthraquinone which is a phenolic compound that acts as a laxative, antimicrobial agent and has a strong analgesic effect. It also has campesterol, sitosterol and lupeol compounds. These compounds act as an anti-inflammatory and antibacterial agent (1,2).

Escherichia coli is the bacteria that most often cause urinary tract infections and nosocomial infections. *E. coli* can cause primary intestinal infection and also used to assess whether a water supply for household use is good or not. *E. coli* is a bacteria commensal which can pathogenic, acting as a primary cause of morbidity and mortality throughout the world (1,3,4).

Chinese lamtoro and petai plants come from tropical America that has a deep root system and resistant to dry conditions. Chinese petai contains alkaloids, saponins, flavonoids, lectins, calcium, phosphorus, iron, tannins, mimosin, leukanin, proteins vitamin A and vitamin B (6). The leaves contain 22% protein (4). Seeds, leaves and all parts of the plant can use to treat several diseases, such as diabetes, fracture, intestinal worms, ulcer, late menstruation, inflammation of the kidneys (nephritis) and insomnia.

Aloe vera is a traditional medicinal plant that has many benefits and benefits. It is a cactus-like plant originating from Africa, which belongs to the Liliaceae family and closed seed plants, can grow in dry areas and also in cold climates (7,8).

E. coli belongs to the family Enterobacteriaceae. This bacterium is a Gram-negative bacterium, in the form of a short stem, has a flagellum, measuring 0.4 to 0.7 µm x 1.4 µm. *E. coli* grows well in almost all breeding media can ferment lactose and is micro-aerophilic. These bacteria become pathogens when outside intestinal tissue. Clinically frequently infected sites are the urinary tract, bile duct, and other places in the abdominal cavity and any anatomic site (e.g. prostate gland, lung, bone) can be the site of disease (9). *E. coli* is a gut flora in the digestive tract that can turn into pathogenic opportunists and cause diseases such as diarrhoea. This bacterium has various pathogenic strains, including Enteropathogenic *Escherichia coli* (EPEC), Enterotoxigenic *Escherichia coli* (ETEC), Enteroinvansive *Escherichia coli* (EIEC) and Enterohemorrhagic *Escherichia coli* (EHEC) (10,11).

Combination therapy uses to broaden the spectrum of antimicrobials, minimize toxicity, slow down the process of antibiotic resistance and reduce the dose of the drug and has considerable antimicrobial activity. Recently, there has been no information about the effective concentration of the combination of extracts of Chinese petai leaves and aloe inhibiting the growth of the bacteria *E. coli*. The purpose of this study

was to analyze the interaction effects of ethanol extracts of Chinese petai leaves and aloe vera leaves in inhibiting the growth of *E. coli* (4,5).

MATERIALS AND METHODS

This research conducted using the completely randomized experimental laboratory method. The research method involves making ethanol extract from *Simplicia* (Crude drugs) by maceration using 96% ethanol solvent. It also examines the interaction between ethanol extracts of Chinese petai leaves and aloe vera stem on the inhibitory power of *E. coli* and in *vitro* by measuring the inhibitory by diffusion method according to *Kirby Bauer*. The principle of the Kirby Bauer method is the inhibition of the growth of microorganisms, the diameter of the zone of inhibition of bacterial growth shows the sensitivity of the bacteria, the wider the zone diameter formed by bacteria, the better it is to inhibit bacterial growth.

The tools used in this study were Petri dishes, tissue cotton, test tubes, wire mesh, syringes, matches, bunsen burner analytical scales, slide callipers, microscopes, sterile stick cotton, Erlenmeyer, beaker glass, parchment paper, tweezers, ovens, incubators, label paper, measuring pipettes, water baths, paper cutters, rotary evaporators. The materials used in this study are pure strains of *E. coli* ethanol extracts of Chinese petai leaves and aloe vera, sterile physiological NaCl (in packaging), steril empty disks, Aquades as negative controls and antibiotic as the positive control. While, the culture media used were MHA (*Muller Hilton Agar*), NB (*Nutrient Broth*), Endo Agar, Nutrient Agar, and Mc Farland Solution.

The extraction method used to extract Chinese petai plants and aloe vera is a cold method extraction method, namely maceration. Chinese petai leaves that turned into Simplicia powder are added with 70% ethanol solvent until submerged. Maceration poured and squeezed. The pulp is macerated again with a new solution until submerged. The maceration process carried out three times then it was evaporated on a *rotary evaporator* until a thick extract of Chinese petai leaf obtained then lastly weighed to determine its weight.

Extraction of Chinese Petai Leaves and Aloe Vera

Aloe vera leaves peeled first to get as much as 1000 grams of aloe vera gel mashed with a blender then soaked with 96% ethanol solvent until aloe vera is submerged completely, after that it is allowed to stand for 2-3 days in a closed jar. Then, the liquid extract was filtered with gauze filter and collected the extract in a bottle. Steering of all filtrates obtained from the immersion. Concentrated with a *rotary evaporator* until thick extract is obtained (1).

Extract Determination

The extract yield calculated by comparing the weight of the ethanol extract obtained with the initial sample weight. The yield obtains through this formula: (extract weight obtained/initial sample weight) x 100%.

RESULTS

Inhibition test of the ethanol extract of Chinese Petai Leaves and Aloe Vera with the disc diffusion method that carried out with various concentrations of the extract.

Table 1. Results of Inhibitory Tests of Chinese Petai Leaf Extract Against *Escherichia coli*

Extract Concentration (mg/mL)	Extract Repetition (mm)			X̄	SD	P. sig
	1	2	3			
25	0	0	0	0	± 0.	0.00
50	0	0	0	0	± 0.	
75	6	6	7	6.3	± 0.58	
100	7	8	8	7.7	± 0.58	
Control (+) *Chloramphenicol*	30	29	30	29.7	± 0.58	

Table 2. Results of Inhibitory Aloe Vera Leaf Extract Against *Escherichia coli* Bacteria (mm)

Extract Concentration (mg/mL)	Extract Repetition (mm)			X̄	SD	P. Sig
	1	2	3			
25	8	10	9	9	1	0.00
50	10	11	10	10.3	0.58	
75	12	12	11	11.7	0.58	
100	14	15	14	14.3	0.58	
Control (+) *Chloramphenicol*	30	28.5	30	29.5	0.87	

Table 3. Test Results of Combination Inhibition of Chinese Petai Leaf Extract and Aloe Vera on *Escherichia coli* (mm)

Extract Concentration (mg / ml)	Extract Repetition (mm)			X̄	SD	P. Sig
	1	2	3			
25	6	7	6	6.3	0.58	0.00
50	8	9	8	8.3	0.58	
75	10	11	10	10.3	0.58	
100	12	12	11	11.7	0.58	
Control (+) *Chloramphenicol*	27	28.5	26	27.2	1.26	

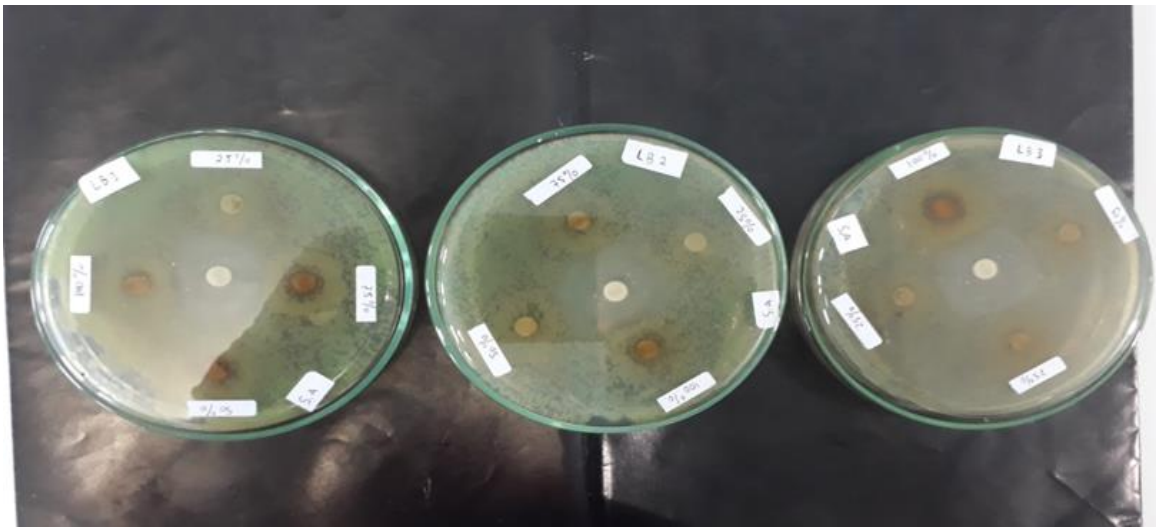

Fig 1. The results of the test for the inhibition of aloe vera against *Escherichia coli* with a concentration of 4 samples. Each sample size was carried out 3 times. In the middle is the (+) chloramphenicol control

Based on Table 1, the inhibitory test results of Chinese Petai leaf extract against *E. coli* (mm) bacteria with a concentration of 75 mg/mL formed inhibition zone 6.3 ± 0.58 mm. Moreover, the amount of 100 mg/mL extract created an inhibition zone of 7.7 ± 0.58 mm. A positive control using the *Chloramphenicol* antibiotic obtained the broadest inhibition zone (29.7 ± 0.58 mm) compared to other concentrations.

Based on Table 2, the inhibitory test results of Aloe Vera extract against *E. coli* (mm) bacteria with a concentration of 25 mg/mL formed inhibition zones 9± 1 mm and it increases following the increase of the extract concentrations. A positive control using the *Chloramphenicol* antibiotic obtained the broadest inhibition zone (29.5 ± 0.87 mm) compared to others.

Based on Table 3, the extract concentration of 25 mg/ml formed an inhibition zone 6.3 ± 0.58 mm, its increases following the increase of the extract concentrations. A positive control using the *Chloramphenicol* antibiotic obtained the broadest inhibition zone (27.2 ± 1.26 mm) compared to others.

DISCUSSION

Interaction of the combination of ethanol extract of Chinese petai leaves and aloe vera against *E. coli* bacteria showed in the *t*est of direct mixing of the two antibacterial agents. It formed the inhibitory zone when placed close together. The test to identify the synergistic effect of antibacterial combinations, discs used and treated with a single antimicrobial agent each, then both are placed at a distance equal to the average number of the radius of the zone of inhibition

of the antimicrobial agent when tested separately (12,4).

The interaction showed the formation of a bridge at or near the intersection of two inhibitory zones or no longitudinal inhibitory zones formed. The combination is said to be synergistic if it created like a bridge at or near the intersection point of two inhibitory zones, or an obstacle to growth which is the combined effect of the two antimicrobial agents (13).

Based on observations of the results of a combination of inhibitory test of Chinese Petai leaf extract and aloe vera on *E. coli* (mm) bacteria, the concentration was 25 mg/mL producing a zone of inhibition 6.3 ± 0.58 mm, a concentration of 50 mg/mL resulted in an inhibition zone of 8, 3 ± 0.58 mm, concentration of 75 mg/mL produces inhibition zone 10.3 ± 0.58 mm, concentration of 100 mg/mL with inhibition zone 11.7 ± 0.58 mm. Positive control using the antibiotic *Chloramphenicol* obtained the biggest inhibition zone compared to other concentrations of 27.2 ± 1.26 mm.

According to Davis stout in A'lana (14), the strength criteria for the combination of Chinese petai leaves and aloe vera concentrations of 25 mg/mL and 50 gr/mL categorized as moderate, at a concentration of 75 mg/mL and 100 mg/mL classified as strong criteria.

Petai China has saponins to inhibit the growth of bacteria *E. coli* by damaging the cytoplasmic membrane in bacteria that cause leakage of metabolites that inactivate the bacterial enzyme systems. Damaged cytoplasmic membranes can prevent the entry of nutrients needed by the nutrients the bacteria produce energy. It causes bacteria to experience growth retardation and even cause bacterial death (6).

Aloe vera contains active substances such as anthraquinone, tannins and flavonoids. Anthraquinone binds with nucleic acids and forms a complex that can damage the acid (DNA and RNA) of inhibited bacteria. Tannin as antibacterial works by inactivating adhesin so that bacteria cannot stick to host epithelial cells. Aloe vera also contains flavonoids, which will cause lysis and inhibit the formation of cell walls. This mechanism causes aloe vera to kill or inhibit the formation of bacteria (15,16,17).

Flavonoids are efficient to inhibit the growth of Gram-positive bacteria. Flavonoids are polar compounds which are easy to penetrate the peptidoglycan layer. Furthermore, the Gram-positive cell wall contains polysaccharides (acidic polysaccharides) is a water-soluble polymer. This cell wall functions as the media of positive ions transport to get in and out. This solubility indicates that the Gram-positive cell wall is polar. Flavonoids disrupt cell wall function. This results in cell lysis (3,20,21).

The mechanism of bacterial inhibition of tannin compounds is by protein reproduction,

ie through reaction with cell membranes, enzyme inactivation and inactivation of genetic material functions, in addition to inhibiting the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot be formed. Saponins which can provide antibacterial activity are avenacin. Saponin compounds will damage the cytoplasmic membrane and kill cells (20,22,23).

CONCLUSIONS

The conclusion of this research is there is an interaction in the combination of ethanol extracts of Chinese petai leaves with aloe vera inhibited the growth of *E. coli*. The most efficient extract concentration was 100 mg/mL. The combination of extracts can provide effective results in inhibiting the growth of bacteria, especially *E. coli* bacteria.

CONFLICT OF INTEREST

There are no conflicts of interest.

ACKNOWLEDGMENT(S)

This study is supported by Ministry of Research, Technology and Higher Education of Indonesia through the research funding for beginner lecturers in 2019.

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