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Original Research Article

From Empirical to Scientific: Microbiological Activity of Black Tea Leaves (*Camellia sinensis* L.) Infusion from Subang Indonesia as Antidiarrhea

Sri Agung Fitri Kusuma[1], Mohammad Nurzaman[2], Johar Arifin[2]
[1]Department of Biology Pharmacy, Faculty of Pharmacy, Padjadjaran University Sumedang, West Java, Indonesia 45363;
[2]Department Of Pharmacy, Al-Ghifari University, Bandung, West Java, Indonesia

***Corresponding author**
Sri Agung Fitri Kusuma
Email: s.a.f.kusuma@unpad.ac.id

Abstract: Subang area is one of the largest tea producers in Indonesia with black tea as a product favored of the Indonesian people. Besides being enjoyed as a drink, empirically black tea leaves infusions were used as an efficacious antidiarrheal. This activity revealed with the fact that black tea leaves were contained simple catechins, examples of which are epicatechin (EC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) which reported can inhibit the growth of many bacterial species. But differences in place of growth may affect secondary metabolites content and microbiological activity. Therefore, the aimed of this study was to evaluate the microbiological activity of black tea leaves that obtained from Subang Indonesia against *Escherichia coli* 0157. The extraction of black tea leaves were prepared using an infusion technique. The secondary metabolites of the extract were analyzed using standard method. The antibacterial activity of the infusion was tested using the disc diffusion method. While the determination of minimal inhibition concentration (MIC) test was conducted using macrodillution method, followed by subculturing the overnight incubation of the MIC test onto Mueller Hinton Agar medium surface, for determining the minimum bactericidal concentration (MBC) value. The results showed that the infusion of black tea leaves had antibacterial activity against *E.coli* with MIC/MBC ranged at 2.5-5.0%w/v.
Keywords: Black tea, *Camellia sinensis*, infusion, *Escherichia coli*, Subang.

INTRODUCTION

Gastroenteritis is a clinico-pathological term that refers to inflammation and oxidative stress of the intestines which leads to disturbance in the balance of secretory and absorptive function of the intestines resulting in diarrhea [1, 2]. Diarrhea is a serious problem affecting 3-5 billion people per year around the world, especially children of below 5 years [3]. The major causative agents of diarrhea in human beings include a variety of enteric pathogenic bacteria such as *Salmonella typhi*, *Shigella flexneri*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Candida albicans* [4, 5]. Due to high mortality and morbidity, especially in children, the WHO together with the United Nations Children's Fund has initiated Diarrhea Disease Control Program to control diarrhea in developing countries. Oral rehydration solution [6], zinc solution [7], probiotics [8], and specific antibiotics have reduced mortality rate in diarrheal disease. However, chronic diarrhea is still a life challenging problem in some regions of the world. Unfortunately, the program does not reach to the needy, and the disease is still a major challenge in front of primary health practitioner as well as researcher. Therefore, the different traditional systems of medicines such as Chinese medicine [9], Japanese medicine [10], acupuncture therapy [11], and ayurvedic medicine [12] are included in this program. 70% of the world population uses traditional and indigenous medicine for their primary health care [3]. Tea is one of chinese traditional medicine that widely used to treat diarrhea.Tea is a traditional beverage originally from China and the second most widely consumed beverage in the world following water. It is brewed from the leaves of *Camellia sinensis* (family: Theaceae). Different types of tea manufactured are: oolong, green, black and Ilex tea depending on the post-harvest treatment and palatability of a particular region [13]. The most kind of tea consumed by many Indonesians people is black tea. Black tea is easy to recognize in the market because of its black color and the most in consumption. Beside that, black tea has many more components than green tea, partly because of the oxidation processes that occur during fermentation. Indonesian tea has a decent quality of export, so it becomes one of the national flagship commodities. West Java is the largest tea producing province in Indonesia. More than 70% of national tea production is produced in this region. Therefore, tea is

one of the leading commodities in West Java after coffee.The plantation area reaches 109,000 hectares or 70% of the area of tea plantations spread in West Bandung regency. The area of tea plantation in Subang (precisely in Ciateur) reaches 3000 hectares. Plantation area is located in the hills on the slopes of the Tangkuban Perahu mountain, so it is expected to produce tea with good quality because it is planted in the highlands. Research for exploring microbiological activity of tea even for the same activity but different sources of ingredients, may affect the outcomes of antimicrobial activity. Therefore, this study was very important to improve the microbiological activity of black tea in infusion, obtained from Subang tea plantation. It is considering that empirically they often used black tea infusion to treat diarrhea. Tea is linked to beneficial effects on human health with the polyphenols as the responsible constituents. In fact, production of secondary metabolites (flavonoids and phenolics) in herbs is related to chalcone synthases activity which is strongly influenced by several parameters such as environmental conditions (light intensity, temperature), nutrient, stress and plant age [14,15].

MATERIALS AND METHODS

Material

The culture media that were used are *Mueller-Hinton Agar* (MHA-Oxoid), and *Mueller-Hinton Broth* (MHB-Oxoid). The chemicals used are distilled water, normal saline solution, barium chloride solution (Merck), sulfuric acid solution (Merck), n-butanol, ferric chloride reagent (Merck), Dragendorf reagents, Lieberman - Burchard reagent, Mayer reagent, technical toluene (Brataco), and vanillin (Merck).

Bacterial Culture

E.coli O157 was obtained from the culture collections in academy of Health analysis Bakti Asih Bandung Indonesia.

Plant Material

The samples that were utilized in this study are *Camellia sinensis* leaves from Subang tea plantation, West Java, Indonesia. Plant sample was identified in Plant Taxonomy Laboratory of Biology Major, Faculty of Mathematics and Natural Science Padjadjaran University.

METHODS

Preparation of Black tea Leaves Infusion

Dried black tea leaves were weighed for 100 g, then put into the erlenmeyer flask, plus sterile distilled water to reach 500 ml, so obtained 20% in concentration. The mixture was then boiled at a temperature of 90^0C - 100^0C.

Phytochemical Screening of Secondary Metabolites

Phytochemical screening of secondary metabolites was using a standard method to determine the contains alkaloids, flavonoids, tannins, saponins, and polyphenol, in infusion of black tea leaves [16].

Preparation of the Bacterial Suspension

At least three to five *E. coli* colonies from an agar plate culture were taken with a loop by touching the top of each colony. The growth is transferred into a tube containing 4 to 5 ml of sterile buffer saline. The turbidity of culture was measured using spectrophotometer until it achieves or exceeds the turbidity of the 0.5 McFarland standards. This results in a suspension containing approximately 1x 10^8 CFU/ml for *E.coli*.

Antibacterial Activity Test

The antibacterial activity of the black tea infusion was done using the agar diffusion methods. The volume of 20 µl standardized cell suspension and 20 ml MHA media at 40-45 ^{0}C was poured in a sterile petri dish, then the mixture was homogenized and allowed to solidify. The infusion was diluted into serial dilution of concentrations using sterile water, as follows: 20%, 10%, 5%, and 2.5%. Then the paper disc was soaked in the infusion water for each concentration and plated on the surface of the agar media. The plates were incubated aerobically at 37°C for 18-24 h. The diameter zones of inhibition were measured using a caliper.

Determination of MIC and MBC Value

MIC value of the *Black tea* leaf infusion was determined using macrodillution broth. The concentration of infusion ranges should be prepared one step higher than the final dilution range required. Then 1 ml extract with concentration 20% ^{w}/v was added into the first tube. Then the volume of 1 ml from the first tube was pipetted then added to the second tube, then the tubes mixed thoroughly and so on until the concentration of every tube was 10%, 5%, 2,5%, 1,25%, 0,62%, 0,31%, 0,15% and 0,075% ^{w}/v. Then 1 ml of bacterial suspension was added to every tube. The liquid media then were incubated at temperature 37^{o}C for 20 h. The MIC value was the lowest concentration (in µg/ml) of an extract that inhibits the growth of *E. coli*. As MBC determination, the loop was dipped into the overnight incubation of MIC tube, and then streaked it on to the agar surface. After that, the plates were incubated at temperature 37^{o}C for 20 h.

RESULTS AND DISCUSSION

Phytochemical Screening Result

Phytochemical analysis revealed that tannins, phenols, and saponin, were present in *C. sinensis* infusion irrespective of the solvent used [Table 1]. Phytochemical analysis suggests the presence of

biologically active compounds in the infusion water of the black tea leaves could be correlated to antibacterial activity against *E. coli.* There are no flavonoid could be detect from the infusion, it might because in tea leaf contains only small amounts of flavonols, such as quercitin, kaempferol, and myricetin [17]. Variation in the chemical constituents in black tea may be attributed to the variation in geographic location of plant specimen like soil and climate.

Table-1: Phytochemical screening

Secondary metabolites	Result
Alkaloids	-
Tannins	+
Flavonoids	-
Saponins	+
Phenols	+

Note: (+) presence; (-) absence

Antibacterial Activity Result

The black tea infusion water showed less activity against *E. coli*. But the increasing of inhibition diameter, which produced by the antibacterial activity of infusion water of black tea leaves against *E. coli*, which is directly proportional to the increasing concentration of the infusion, showed that the infusion has antibacterial effect. The diameter data can be seen in figure 1 and table 2.

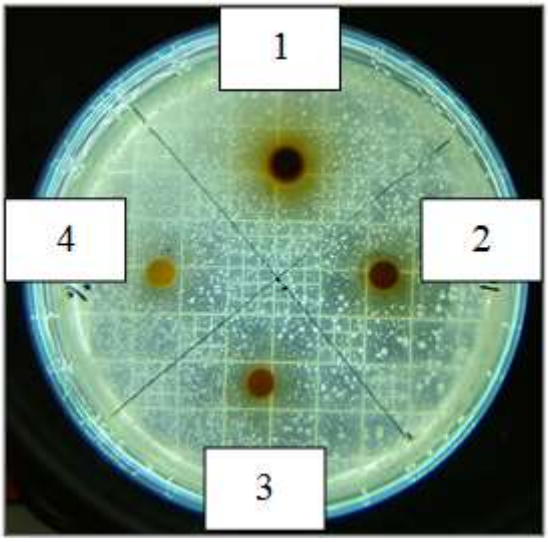

Fig-1: Antibacterial activity result

Note: various concentration of infusion, (1) 20%; (2) 10%; (3) 5%; (4) 2.5%

Table-2: Antibacterial activity results

Concentration (%w/v)	Inhibitory zone diameter (mm)
2.5	0
5	2.5
10	4.0
20	6.0

Note: disc diameter = 2 mm

The antibacterial activity of black tea infusion against *E. coli* was presumably contributed by its secondary metabolites. Tannins can inhibit both glucosyl transferace (GFT) activity and bacterial growth by their strong iron-binding capacity [18]. Polyphenol can cause cell membrane disruption and prevent DNA super coiling eventually leading to bacterial destruction [19]. While saponins possess detergent-like properties and might increase the permeability of bacterial cell membranes without destroying them [20].

MIC and MBC Determination Result

Minimum inhibitory concentrations (MIC) refer to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. The result of MIC then subculturing onto surface of agar media, determined as MBC value, which can be seen in Table 3.

Table-3: Minimum bactericidal concentration value

Infusion concentration (%^{w}/v)	Bacterial growth
20	-
10	-
5	-
2.5	+
1.25	+
0.625	+
0.3125	+
0.15	+
0.075	+

Note: (+) = colony absence; (-) = colony presence

The infusion showed the value of MBC ranged between 2.5 and 5 %^{w}/v. The MIC is a measure of the potency of an antimicrobial effect. The smaller the MIC value, then the drug is said to be more effective.

CONCLUSION

Our results demonstrated that the empiric data about infusion water of *black tea* leaves as antidiarrheal gave the same conclusion by scientific study that the infusion of black tea leaves from subang can be used as antidiarrheal, especially against *E.coli*.

REFERENCES

1. Chatterjee R, Mysore A, Ahya K, Shrikhande D, Shedabale D. Utility of sonography in clinically suspected Dengue. Pediatric Infectious Disease. 2012 Sep 30; 4(3):107-11.
2. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgeman M. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. Journal of pediatric gastroenterology and nutrition. 2012 Jan 1; 54(1):136-60.
3. Mishra A, Seth A, Maurya SK. Therapeutic significance and pharmacological activities of antidiarrheal medicinal plants mention in Ayurveda: A review. Journal of Intercultural Ethnopharmacology. 2016 Jun; 5(3):290.
4. Teke GN, Kuiate JR, Ngouateu OB, Gatsing D. Antidiarrhoeal and antimicrobial activities of Emilia coccinea (Sims) G. Don extracts. Journal of ethnopharmacology. 2007 Jun 13; 112(2):278-83.
5. Kitaoka M, Miyata ST, Unterweger D, Pukatzki S. Antibiotic resistance mechanisms of Vibrio cholerae. Journal of medical microbiology. 2011 Apr 1; 60(4):397-407.
6. Casburn-Jones AC, Farthing MJ. Management of infectious diarrhoea. Gut. 2004 Feb 1;53(2):296-305.
7. Lamberti LM, Walker CL, Chan KY, Jian WY, Black RE. Oral zinc supplementation for the treatment of acute diarrhea in children: a systematic review and meta-analysis. Nutrients. 2013 Nov 21; 5(11):4715-40.
8. Hickson M. Probiotics in the prevention of antibiotic-associated diarrhoea and Clostridium difficile infection. Therapeutic advances in gastroenterology. 2011 May; 4(3):185-97.
9. Leung WK, Wu JC, Liang SM, Chan LS, Chan FK, Xie H, Fung SS, Hui AJ, Wong VW, Che CT, Sung JJ. Treatment of diarrhea-predominant irritable bowel syndrome with traditional Chinese herbal medicine: a randomized placebo-controlled trial. The American journal of gastroenterology. 2006 Jul 1; 101(7):1574-80.
10. Yamakawa JI, Moriya J, Takeuchi K, Nakatou M, Motoo Y, Kobayashi J. Significance of Kampo, Japanese traditional medicine, in the treatment of obesity: basic and clinical evidence. Evidence-Based Complementary and Alternative Medicine. 2013 Apr 15;2013.
11. Manheimer E, Wieland LS, Cheng K, Li SM, Shen X, Berman BM, Lao L. Acupuncture for irritable bowel syndrome: systematic review and meta-analysis. The American journal of gastroenterology. 2012 Jun 1; 107(6):835-47.
12. Mishra A, Sharma V, Hem K, Maurya SK. Plants used for treatment of diarrhea: An Ayurvedic prospective. Innov J Ayurvedic Sci. 2015; 3(1):1-6.

13. Soni RP, Katoch M, Kumar A, Ladohiya R, Verma P. Tea: Production, Composition, Consumption and its Potential an Antioxidant and Antimicrobial Agent. International Journal of Food and Fermentation Technology. 2015 Dec 1; 5(2):95.
14. Ghasemzadeh A, Nasiri A, Jaafar HZ, Baghdadi A, Ahmad I. Changes in phytochemical synthesis, chalcone synthase activity and pharmaceutical qualities of Sabah snake grass (Clinacanthus nutans L.) in relation to plant age. Molecules. 2014 Oct 30; 19(11):17632-48.
15. Lillo C, Lea US, Ruoff P. Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. Plant, cell & environment. 2008 May 1; 31(5):587-601.
16. York WS, Darvill AG, McNeil M, Stevenson TT, Albersheim P. Isolation and characterization of plant cell walls and cell wall components. Methods in enzymology. 1986 Dec 31;118:3-40.
17. Kirk RE. Othmer; DF Encyclopedia of Chemical Technology, Vol. 14. The Interscience Encyclopedia Inc., New York. 1953:316.
18. Anita P, Sivasamy S, Kumar PM, Balan IN, Ethiraj S. In vitro antibacterial activity of Camellia sinensis extract against cariogenic microorganisms. Journal of basic and clinical pharmacy. 2014 Dec; 6(1):35.
19. Goenka P, Sarawgi A, Karun V, Nigam AG, Dutta S, Marwah N. Camellia sinensis (Tea): Implications and role in preventing dental decay. Pharmacognosy reviews. 2013 Jul 1; 7(14):152.
20. Jacob MC, Favre M, Bensa JC. Membrane cell permeabilisation with saponin and multiparametric analysis by flow cytometry. Cytometry. 1991 Jan1;12(6):550-8.