

THE ANTIBACTERIAL EFFECTS OF ESSENTIAL OIL FROM GALANGAL RHIZOME *ALPINIA GALANGA* (LINN.) PIERREON RAT (*RATTUS NORVEGICUS* L.) WERE INFECTED BY *SALMONELLA TYPHI*

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ABSTRACT

Objectives: The research was aimed to assess the effect of essential oil of Galangal rhizome (*Alpinia galanga* (Linn.) Pierre.) in vivo by Widal test using mice infected with *Salmonella typhi*.

Methods: Antibacterial activity of the essential oils was determined by Widal test for measuring antibody titre of Salmonella antigen. Study was begun by isolating the essential oils galangal rhizome (*Alpinia galanga* (Linn.) Pierre.) followed by tested the antibacterial activity of *Salmonella typhi* in rats (*Rattus norvegicus* L.). Mice were divided into six treatment groups, ie normal control group, negative control group given Gum Arabic 10%, positive control group given the suspension of chloramphenicol 8 mg/250 g BW, experimental group 1 given the emulsion of essential oil at the dose 25 mg / ml, experimental group given the emulsion of essential oil at the dose 50 mg / ml, and the experimental group 3 given the emulsion of essential oil at the dose 75 mg / ml. Before treated, mice were adapted for 2 weeks and measurement of antibody titer (Widal test 1) as a baseline, then the mice induced by *Salmonella typhi* suspension oral. On day 8 titre antibody was measuring (Widal test 2) and followed the administration of the test material orally 3 times a day for 7 consecutive days - respectively. On day 14 measurements of antibody titer was repeated (Widal test 3) to see the effectiveness of treatment.

Results: An essential oil from Galangal (*Alpinia galanga* (Linn.) Pierre.) at the dose 25mg/ml, 50 mg/ml and 75 mg/ml can reduce antibody titer of Salmonella antigen.

Conclusion: The results obtained in the study show that essential oils from galangal rhizome possess antibacterial effect against *Salmonella typhi*.

Keywords: Antibacterial effects, Essential oil, Galangal rhizome, *Salmonella typhi*, Widal test.

INTRODUCTION

Typhoid fever is an acute infectious disease of the small intestine with symptoms of fever a week or more with disorders of the gastrointestinal tract with or without impaired consciousness. The disease is caused by *Salmonella typhi* or *Salmonella typhosa*. Transmission of the disease is almost always occurred through contaminated food and drink [1]. The pathophysiology of typhoid fever is a complex process which proceeds through several stages. The disease begins with an asymptomatic incubation period of 7-14 days, (inversely related to the size of the infecting dose), during which bacteria invade macrophages and spread throughout the reticuloendothelial system. The 1st week of symptomatic disease is characterized by progressive elevation of the temperature followed by bacteremia. The 2nd week begins with the development of rose spots, abdominal pain, and splenomegaly [2].

Treatment of typhoid disease intended to inhibit the growth of bacteria *S. typhi*. It is recommended that treatment of typhoid fever begin on the basis of clinical findings before definitive diagnosis. Unfortunately, in endemic regions, facilities for definitive diagnosis, based on blood or bone marrow culture or serologic tests may be entirely lacking. Supportive measures such as oral or intravenous rehydration, antipyretics, appropriate nutrition and blood transfusion are important [3]. A synthetic antimicrobial drug which is the first line antibiotics for the treatment of typhoid fever is chloramphenicol, trimethoprim-sulfamethoxazole, and ampicillin/amoxicillin. In addition, the second line drugs used in the treatment of typhoid fever are the fluoroquinolones (ciprofloxacin, norfloxacin, pefloxacin, and ofloxacin) the third generation cephalosporins (ceftriaxone, cefotaxime,

cefoperazone, and cefixime), and other antibiotics (aztreonam and azithromycin) [4].

Treatment with a synthetic antimicrobial constrained by the high price of medicines and the high incidence of drug side effects. The non-availability and high cost of new generation antibiotics with limited effective span have resulted from an increase in morbidity and mortality. Various attempts were made in the search for antimicrobial, one of which is derived from natural materials. Utilization of traditional medicine has been known since the first and passed down from generation to generation because it is considered safer and cheaper than modern medicine [5].

The traditional system of medicinal consists of a large number of plants with various medicinal and pharmacological importances and hence represents a priceless tank of new bioactive molecules. *Alpinia galanga* (Linn.) Pierre is one among these, found all over the world and has been recognized in the different traditional system of medicines for the treatment of various diseases. Different parts of this plant are traditionally claimed to be used for the treatment of ailments including antimicrobial, anti-fungal, anti-tumor, antihelminthic, anti-diuretic, anti-ulcerative, disease of the heart, rheumatic pains, chest pain, dyspepsia, fever, diabetes, burning of liver, and kidney disease [6].

One of the medicinal plants that can inhibit the growth of *S. typhi* is the galangal rhizome (*A. galanga* [Linn.] Pierre). *In-vitro* test conducted by Fardiaz 1999 show galangal rhizome can inhibit the growth of bacteria *S. typhi* at a dose of 25 mg/ml with an average diameter of the zone of inhibition 32.5 mm [7]. The essential oils of the rhizome of *A. galanga* showed antimicrobial activity [8]. Essential oil had shown significant

activity against *Staphylococcus aureus*, *Streptococcus suis*, *Erysipelothrix rhusiopathiae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Pasteurella multocida* and *Arcanobacterium pyogenes*, the effects were attributed to 1,8-cineole, 4-allylphenyl acetate, and α -bisabolene [9].

This study was conducted to assess the effect of essential oil of galangal rhizome (*A. galanga* [Linn.] Pierre.) *in vivo* by Widal test using mice infected with *S. typhi*. Thereby using of galangal rhizome can be proven scientifically as antityphoid.

METHODS

Material

Ginger rhizome (*A. galanga* [Linn.] Pierre.) was obtained from Balitro and has determined in Bogoriense Herbarium, Botanical Research and Development Center, Indonesian Institute of Sciences, Jakarta, rat (*Rattus norvegicus* L.), *S. typhi* bacteria was obtained from the Central Health Laboratory, Jakarta, chloramphenicol, and aqua distillate.

Methods

Isolation of essential oils

Ginger rhizome aged 2.5-4 months was taken entirely, then washed with running water to clean and drained following dried by drying without direct sun. Then, put into a grinding machine, chopped to pieces obtained a bit rough. The rough rhizome weighed as much as 7 kg and put in a steam-water distillation equipment for approximately 5 hrs. Collect the filtrate and add Na_2SO_4 eksikatus to absorb water, so the pure essential oil obtained from the rhizomes of galangal [10]. Essential oils were then examined using a spectrophotometer gas chromatography-mass spectrometry (GC-MS).

Preparation of *S. typhi* bacteria suspension

S. typhi bacteria suspension made using physiological NaCl of bacteria that have been rejuvenated earlier so obtained the age of bacterial culture 18-24 hrs. Suspension density adjusted to the standard solution of 1 McFarland corresponds to 3×10^8 bacteria/ml [11].

Anti-typhoid test

Animal and study design

Thirty-six adults, male Sprague-Dawley rats (aged 3 months), weighing 200-280 g were obtained from the Animal Source Unit, Indonesia University. The rats were randomly assigned into five groups (two control and three experimental groups [EG]) comprising of five animals each. Prior study approval was obtained from the Ethics Committee Medical Faculty of Indonesia University. All animal management and procedures were performed in accordance with the recommended guidelines. The rats were kept in stainless-steel cages and maintained at room temperature of $27 \pm 2^\circ\text{C}$ with a 12 hrs light-dark cycle. All rats had free access to food and water *ad libitum* during the study period.

Typhoid fever was induced experimentally by 1 ml suspension of *S. typhi* orally [12]. Every day for 7 day's body temperature measured with a thermometer. The incubation period is 7-10 days and on the 7th day body temperature will increase. On day 8-14, each treatment group was given the test material. The normal control group (NoCG) did not give anything, The negative control group was given gum Arabic 10%, positive control group (PCG) was given antibiotic chloramphenicol 8 mg/250 g body weight (BW), EG1 was given 25 mg/ml emulsion of essential oils, EG2 was given 50 mg/ml emulsion of essential oils and EG3 were given 75 mg/ml emulsion of essential oils. Each test material administered 3 times daily.

Blood collection

Blood collection carried out before induction of *S. typhi* (day 0), day 7 and day 14 through vena caudal. Blood was collected and then centrifuged to obtain plasma and later store at -70°C for analysis Widal test [13].

Table 1: GC-MS

Number of peak	Retention time	Compounds suspected
1	5.83	1,8-cineole
2	9.25	Terpinen-4-ol
3	9.61	(-)- α -terpineol
4	13.57	4-allylphenyl acetate
5	14.48	2,6-octadien-1-ol 3,7-dimethyl-acetate
6	15.44	β -caryophyllene
7	16.28	1,6,10-dodecatriene 7,11-dimethyl-3-methylene
8	17.00	1-pentadecene
9	17.40	1-pentadecene
10	17.62	β -bisabolene
11	21.21	6,8-heptadecadiene
12	21.38	8-heptadecene
13	21.66	8-heptadecene

GC-MS: Gas chromatography-mass spectrometry

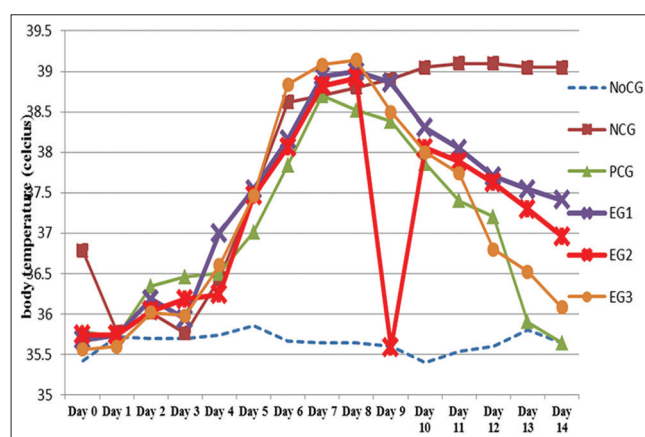


Fig. 1: Body temperature ($^\circ\text{C}$) changes for 14 days

Data analysis

Data obtained are presented in tabular form. Data was analyzed with Anova using SPSS 20 for windows.

RESULTS

Isolation of essential oils from ginger rhizome produces essential oil with a yield of 0.03%. Essential oils have characteristic: Yellow clear, distinctive smell and taste bitter and spicy on the tongue. Results of GC-MS are shown in Table 1.

Rats body temperature changes after giving suspension bacteria can be shown in Table 2.

Body temperature changes for 14 days can be seen in Fig. 1. Widal test results (mean value of titer O and H antigen) can be shown in Table 3. The increase in titer more than 1/160 shows that rats suffering typhoid fever.

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DISCUSSION

A. galanga (L.) Willd., family Zingiberaceae commonly referred to as galangal, is widely cultivated in South-east Asian countries such as Philippines, Indonesia, Thailand, India, and China [14]. *A. galanga* has been thoroughly studied by various workers and chemical

Table 2: Rats body temperature changes after giving suspension bacteria

Preliminary test	Changes in body temperature of the mice (day)										
	0	1	2	3	4	5	6	7	8	9	10
I	35.6	35.6	35.6	35.7	35.6	35.6	35.7	35.7	35.6	35.7	35.7
II	35.5	35.6	35.8	35.9	36.3	36.5	36.7	36.9	37.0	37.5	37.9
III	35.2	35.3	35.6	35.9	36.6	37.0	37.6	37.8	38.0	38.5	38.6
IV	35.5	35.5	36.2	37.1	37.6	38.0	38.3	38.9	39.0	39.2	39.5

Preliminary test I: Induced 0.5 ml bacterial suspension at a concentration of 0.5 McFarland, Preliminary test II: Induced 1 ml bacterial suspension with a concentration of 0.5 McFarland, Preliminary test III: Induced 0.5 ml bacterial suspensions with a concentration of 1 McFarland, Preliminary test IV: Induced 1 ml bacterial suspension at a concentration of 1 McFarland

Table 3: Widal test results (mean value of titer O and H antigen)

EG	Test Widal I (Day 0)		Test Widal II (Day 7 after induction of <i>S. typhi</i>)		Test Widal 3 (Day 14 after induction of <i>S. typhi</i>)	
	Antigen O	Antigen H	Antigen O	Antigen H	Antigen O	Antigen H
NoCG	Negative	Negative	Negative	Negative	Negative	Negative
NCG	Negative	Negative	1/160	1/160	1/160	1/160
PCG	Negative	Negative	1/176	1/160	Negative	Negative
EG1	Negative	Negative	1/224	1/192	1/80	1/48
EG2	Negative	Negative	1/224	1/224	1/56	1/40
EG3	Negative	Negative	1/160	1/160	1/40	1/40

EG: Experimentation group, NoCG: Normal control group, NCG: Negative control group, PCG: Positive control group

constituents have been isolated such as galangal flavonoid [15], phenylpropanoids and hydroxybenzaldehyde (1'S-1'-acetoxychavicol acetate and 1'S-1'-acetoxy eugenolacetate) [16], hydroxy-1,8-cineole glucopyranosides, (1R, 2R, 4S)-and (1S, 2S, 4R)-trans-2-hydroxy-1,8-cineole β -D-glucopyranoside, and (1R, 3S, 4S)-trans-3-hydroxy-1,8-cineole β -D-glucopyranoside, which are possible precursors of acetoxy-1,8-cineole [17]. Main compounds of galangal extract are 1,8-cineole, β -bisabolone and β -selinene. α -selinene, farnesene, 1,2-benedicarboxylic acid, germacrene B, and pentadecane are the minor components [18]. Studied by Wu *et al.*, 2014 showed that the main components of the essential oil were identified to be eucalyptol (22.63%), (1S)-(1)- β -pinene (14.36%), 1R- α -pinene (10.89%), α -terpineol (8.59%), and L(-)-born [19].

From the results of preliminary experiments, it is known that the administration of 0.5 ml bacterial suspension with a turbidity of 0.5 McFarland showed no increasing in titer and rat body temperature. Giving a bacterial suspension with a concentration of 0.5 McFarland, induction volume of 1 ml, there was an increase in titer on *S. typhi* O and H of 1/40 and rat body temperature reaches 36.9°C, these mice showed symptoms of suffering from typhoid. Giving a bacterial suspension with a concentration of 1 McFarland, induction volume of 0.5 ml titer rise in *S. typhi* O and H, respectively - 1/80 and 1/40, respectively, and rat body temperature reaches 37.8°C showed mice still suffer symptoms of typhus. Giving a bacterial suspension with a concentration of 1 McFarland with an induction volume of 1 ml showed a titer rise in *S. typhi* O and H, respectively, - each 1/320 and 1/160 and rat body temperature reaches 39.5°C showed mice suffering from typhus.

Administration of 1 ml bacterial suspension with a concentration of 0.5 McFarland increasing titer *S. typhi* O and H of 1/40 and rat body temperature reaches 36.9°C, while the administration of 0.5 ml bacterial suspension with a concentration of 1 McFarland increasing titer *S. typhi* O and H, respectively, - each 1/80 and 1/40 and rat body temperature reaches 37.8°C and administration of 1 ml of a bacterial suspension with a concentration of 1 McFarland increases titer *S. typhi* O and H, respectively, - each 1/320 and 1/160 and rat body temperature reaches 39.5°C.

The clinical presentation of typhoid fever varies from a mild illness with low-grade fever, malaise and dry cough to a severe clinical picture with abdominal discomfort, altered mental status, and multiple

complications. Clinical diagnosis is difficult. In the absence of laboratory confirmation, any case of fever of at least 38°C for 3 or more days is considered suspect if the epidemiological context is suggestive [1].

Fever is a natural reaction during a number of illnesses. In several cases, the absence of the natural reaction is a more alarming sign than the presence of fever itself. Fever is usually accompanied by different general symptoms, such as sweating, chills, the sensation of cold and other subjective sensations. Causes of fever include infections caused by parasites, viruses, bacteria, rickettsia, chlamydia, immune reactions (including the defects in collagen, immunological abnormalities, and acquired [20,21]). The febrile response is a complex physiologic reaction to disease involving a cytokine-mediated rise in body temperature, generation of acute-phase reactants, and activation of numerous endocrinologic and immunologic systems [21,22].

Cells stimulated by exogenous pyrogens centrally like *S. typhi* bacteria affect the thermosensitive neurons in the preoptic area of the hypothalamus, increase the production of heat and decrease in heat loss. The body temperature increases until it reaches the set point. This information is transferred by the temperature of blood that flows around the hypothalamus. The decrease of temperature is controlled by activation of mechanisms regulating increased outcome of heat to the surrounding area. Increased outcome continues in the favorable case until the new equilibrium is achieved [23].

Sources of pyrogens, substances that cause fever, are both exogenous and endogenous. The most common exogenous sources are microorganisms, their products or toxin such as lipopolysaccharide endotoxin of Gram-negative bacteria like *S. typhi*. Exogenous pyrogens induce formation and release of endogenous pyrogens or pyrogens cytokines. The most common pyrogen cytokines are interleukin-1 α (IL1 α) and 1 β , tumor necrosis factor α , IL6, ciliary neurotropic factor and interferon γ [20]. The interferons also display pyrogenic activity. Pyrogens, prostaglandin E₂ (PGE₂) release and hypothalamus play major roles in the pathophysiology of fever mechanism [23]. All pathogenic Salmonella species, when present in the gut are engulfed by phagocytic cells, which then pass them through the mucosa and present them to the macrophages in the lamina propria. With toll-like receptor (TLR)-5 and TLR-4/MD2/CD-14 complex, macrophages recognize pathogen-associated molecular patterns such as flagella and lipopolysaccharides (LPSs). Macrophages and intestinal epithelial cells then attract T-cells

and neutrophils with interleukin 8 (IL-8), causing inflammation and suppressing the infection [24].

Blatteis, 2007 report that LPS-induced fever arises as the result of a complex, phased sequence of interactions among soluble factors and cells that is initiated in the periphery and then transmitted neurally to the ventromedial preoptic area (VMPO), which modulates the febrile response. Peripheral PGE₂ rather than pyrogenic cytokines initiates the febrile response, NE propagates the pyrogenic signal forward within the VMPO, NO modulates its release in the VMPO and, hence, the intensity of the febrile response, and cyclooxygenase (COX-2)/microsomal PGE synthase-1-dependent PGE₂ mediates the late, but not the early phase of fever; the latter appears to be independent of COX-derived PGE₂ [25].

Typhoid fever is caused by *S. typhi*, a Gram-negative bacterium. The incubation period is usually 8-14 days, but may range from 3 days up to 2 months. The clinical presentation of typhoid fever varies from a mild illness with low-grade fever, malaise and dry cough to a severe clinical picture with abdominal discomfort, altered mental status and multiple complications [26]. There are a number of tests available nowadays from molecular to immunological and biochemical to microbiological [27].

Widal is a serological diagnosis test for enteric fever. It is an agglutination reaction demonstrating the presence of LPS somatic (O) and flagella (H) agglutinins to *S. typhi* in the serum of a patient using suspensions of O and H antigens. The Widal test had been to increase the index of suspicion for the presence of typhoid fever by demonstrating a positive agglutination during the acute and convalescent period of infection with evidence of a four-fold rise in antibody titre [28].

Interpretation of Widal test must be considered some factors, such as the timing of the test, as antibodies begin to arise during the end of the 1st week. The titer increases during the 2nd, 3rd and 4th week after which it gradually declines. The test may be negative in the early part of the first week. A single test is usually not much value. A rise in titer between two sera specimens is more meaningful than a single test. If the first sample is taken late in the disease, a rise in titer may not be demonstrable. Instead, there may be a fall in titer. Baseline titer of the population must be known before attaching significance to the titers. The antibody levels of individuals in a population of a given area give the baseline titer. A titer of 100 or more for O antigen is considered significant and a titer in excess of 200 for H antigens is considered statistically significant [28,29].

A single Widal test is still a useful diagnostic tool in typhoid fever. An "O" titer in isolation, an "H" titer in isolation and an "O" or "H" titer when considered together, or more than or equal to 1:160, with relevant clinical findings, was found to be highly suggestive of typhoid fever [30].

Result of Widal test 1 show that it does not see increasing in O and H antigen titer because the mice did not infected with *S. typhi* yet, while the results of Widal test II mice have induced 7 days with *S. typhi*, show enhancement in antigen titer. Widal test III results revealed a decrease in titer of antigen in the PCG were treated with chloramphenicol at a dose of 8 mg/250 g BW, emulsions essential oil at a dose of 25, 50 and 75 mg/ml noticeable decrease in titer at each group: Negative titer in CPG, 1/40 in EG3 and 1/80 in EG1 and EG2.

An *in-vitro* study conducted by Fardiaz 1999 showed that the rhizome of ginger can inhibit the growth of bacteria *S. typhi* at a dose of 25 mg/ml had an average of inhibition zone of 38.3, 32.3, and 32.5 mm [7]. *In-vivo* tests using rats induced *S. typhi* at a dose of 25 mg/essential oils from ginger rhizome can inhibit the growth of bacteria. This can be seen from the reduction in titer after administration of the treatment from 1/160 into 1/80. This shows that at doses of 25 mg/ml can inhibit the growth of bacteria.

Essential oils from family Zingiberaceae are found to be rich source of terpenes monoterpenes and sesquiterpenes and phenols. The

relation between terpenes as antimicrobials are well-established and studies have demonstrated optimal potential of these essential oils for antimicrobial activity [31]. *A. galanga* oil had antibacterial action probably as a result of its modification of the bacterial cell membrane, disrupting the membrane's permeability. It has been found that the presence of diterpenes and sesquiterpenes in crude extract plays a significant role for producing antibacterial activity [32].

Fig. 1 shows that the maximum rise in body temperature occurred on day 8 to EG1, EG2, and EG, respectively, are at 39.0°C, 38.92°C, and 39.14°C. In the NoCG maximum body temperature increase on the 7th day of 38.7°C. Administration of essential oils for 7 days 3 times daily at a dose of 75 mg/ml (EG3) is able to lower the body temperature better than the group a dose of 50 mg/ml (EG2) and 25 mg/ml (EG3) each 36.08°C, 36.96°C, and 37.41°C, respectively.

A study conducted by Reddy *et al.*, 2011 showed that *A. galanga* leaf methanol extract exhibited significant activity against *Pseudomonas* and was quite comparable with the standard antibiotic gentamicin sulfate. The leaf ethyl acetate extract showed pronounced inhibitory activity against *S. aureus* and the activity was comparable with the standard antibiotics ofloxacin, ciprofloxacin, and tobramycin. The minimum inhibitory concentration (MIC) of methanol and ethyl acetate extracts of the leaves of *A. galanga* was found to be 0.5 mg/ml [33].

Results from study, the antimicrobial potential of variety of extraction of *A. galanga* extract such as hexane, ethyl acetate, ethanol, and the essential oil, respectively, that against swine pathogenic bacteria compose of *E. coli*, *S. aureus*, *Salmonella typhimurium*, *Salmonella enteritidis* and *P. multocida* showed that essential oil of *A. galanga* have the best antibacterial and bactericidal activities with MIC and minimum bactericidal concentration to *E. coli*, *S. aureus*, *S. typhimurium* and *S. enteritidis* at 8 mg/cc and to *P. multocida* at 16 mg/cc [34].

Essential oil from *A. galanga* had shown significant activity against *S. aureus*, *S. suis*, *E. rhusiopathiae*, *P. aeruginosa*, *E. coli*, *P. multocida* and *A. pyogenes*, the effects were attributed to 1,8-cineole, 4-allylphenyl acetate, and α -bisabolene [35]. *A. galanga* caused both outer and inner membrane damage, and cytoplasm coagulation. The disruption of the cytoplasmic membrane properties was determined by the releasing of cell materials including nucleic acids [9].

Results from statistical test showed that no significant difference antityphoid activity between positive control and essential oil from *languas galangal* concentration of 25 (EG1), 50 (EG2) and 75 mg/ml (EG3); $p > 0.05$. The best concentration essential oils from *languas galangal* for antityphoid activity is 75 mg/ml.

CONCLUSION

Administration of essential oil of from *A. galanga* (Linn.) Pierre rhizome at the dose 25, 50, and 75 mg/ml have antityphoid effect on rats that induced with *Salmonella typhi*.

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