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Characterization of Cinnamadehyde Compound Isolated from Cinnamon Oil and Its Salmonella Typhy Antibacterial Activity

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ABSTRACTS

Today many infectious diseases are common. All of the diseases are caused by agents such as viruses or bacteria which are pathogenic and *Salmonella typhi* is one of the pathogenic bacteria. In addition, many antibiotics are not able to work properly because of the resistance of bacteria against the exciting antibiotics. Therefore, research to discover the new anti-bacterial compounds derived from natural materials is important to do. This research was conducted in several stages, that involved: (1) Isolation of cinnamaldehyde from cinnamon oil by two methods: thin layer chromatography using n-hexane: ethanol eluent at a ratio of 5 : 95 and bisulfate additional, (2) Identification of cinnamaldehyde by UV-Vis Spectrophotometer and GC-MS to define its purity, (3) The various concentrations of the cinnamaldehyde extracts were incubated with *Salmonella typhi* bacteria to evaluate the antibacterial activities of the extracts, (4) The growth of the tested bacteria was observed using a colony counter to see the diameter of the resistance which was caused by the test solution. Analysis result of Gas Chromatography – Mass Spectrophotometer showed that cinnamon oil (Cassia oil) contains cinnamaldehyde as the main component of 88.33 %. The isolation of cinnamaldehyde by bisulfate addition method obtained cinnamaldehyde as yellow fluid with a yield of 86.79 %. The antibacterial activity test indicated that cinnamaldehyde had the potential as an antibacterialagainst the *Salmonella typhi which was* known from its inhibition zone. At the concentration of 100 ppm, cinnamaldehyde solution showed an inhibitory diameter of 15.4 mm and amoxicillin antibiotic showed of 16.6 mm.

Keywords: activity, antibacterial, cinnamaldehyde, isolation, characterization, inbihitory zone

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1. Introduction

An antibiotic is given for the treatment of diseases or infections caused by bacteria. Not only bacteria, target organisms of antibacterial also include fungi and other parasites (Nordqvist, 2009). Research on antibiotics continues to evolve over time and get the more attention of the researchers. This is related to the role of antibiotics in reducing death rates caused by infectious diseases. Some dangerous diseases caused by bacteria are tuberculosis, pneumonia, meningitis, typhoid, cholera, diarrhea, tetanus and others.

Research on discovery of new antibiotic have been succeed to develop some effective antibiotic to against various types of bacteria but in the other side there are also strains of bacteria that are resistant to exiting antibiotics (Suwandi, 1992). The emergence of this resistance caused by bacteria can adapt to the presence of antibiotics in clinical concentration and also can be caused by the wrong usage of antibiotic by patient. Various studies discovered that 40-62 % caused by usage of antibiotic for the diseases that not require antibiotics (Jumina, 2015).

Some antibiotics that have been resistant as reported (Rao 2012, 2015) are chloramphenicol (*P.aeruginosa, K. pneumoniae, E. coli, S. typhimurium, V. cholerae*), macrolides (*Streptococcus pneumoniae, Enterococcus sps, Bacteroides sps, Pseudomonas sps* and Enterobacteriaceae), tetracyclines (*S. aureus, E. coli, A. baumannii, S. typhimurium*), aminoglycosides (*E. coli, P. aeruginosa, A. baumannii*) and also beta-lactams (*H. influenzae, P. aeruginosa, A. baumannii*). In addition, there was increasing of 440,000 new cases due to multidrug-resistant tuberculosis (MDR-TB) each year which causes at least 150,000 cases of death each year. Indonesia ranked eighth out of 27 high MDR load countries (WHO, 2009).

There is two ways to develop new antibiotics that are: 1) Isolation of the active compounds in medical plants that traditionally used to treat diseases caused by bacterial and 2) synthesized the groups of compounds that have been known to have antibiotic activity. Economically, the first way is advantageous if the antibacterial content in plans or microorganismis present in large quantities. However, if the

antibacterial content is presented in small quantities the second way will be more profitable. One of the medicinal plants that has been proven to have antibiotic activity is *Garcinia* (the mangosteen group) which is well known as a secondary metabolite source of phenolic compounds, especially xanthones (Likhitwitayawuid, 1998; Syamsudin *et al*, 2008; Chaveri *et al.*, 2008). Xanton group compounds have been proven to have activity as antibiotics, anti-malarial, anti-cancer, anti-HIV, anti-oxidants and anti-inflammatory (Ito *et al.*, 1998; Lembege *et al., 2008*; Riscoe *et al*, 2005). Despite having a large antibiotic activity, however, the isolation process of xanton yields only a very small isolation rendement, which is about 0.55%. Therefore, efforts to discover antibacterial substances in other plants are considered important.

One of the compounds that are high content in cinnamon plants is cinnamaldehyde. The content of cinnamaldehyde in cinnamon oil that isolated fromcinnamon plants from three different regions in East Nusa Tenggara (NTT) which are from Timor Tengah Selatan (TTS), Sumba Barat Daya (SBD), and Ende are quite high: 83.65%, 60.85%, and 80.85% respectively (Budiana, 2016).Cinnamaldehyde is also found to have activity as a sunscreen (Budiana and Rima, 2015). The most obvious application for cinnamaldehyde is as flavoring in chewing gum, ice cream, candy, and beverages. It is also used in some perfumes of natural, sweet, or fruityscents. Almond, apricot, butterscotch, and other aromas may partially employ the compound for their pleasant smells. Cinnamaldehyde is also used as a fungicide that inhibits the growth of mold. It's low toxicity and well-known properties make it ideal for agriculture. Cinnamaldehyde is an effective insecticide, and its scent is also known to repel animals, such as cats and dogs.

Cinnamaldehyde with the chemical formula C_9H_8O has a molar mass of 132,16 g/mol, sharp smelled with the density of 1.0497 g/mL and a structure as shown in Fig.1. The melting point of this compound is -7.5 ^oC (18.5 ° F; 265, 6 K), and a boiling point of 248 °C (478 ° F; 521 K). This compound dissolves well in ether, chloroform, petroleum ether, and alcohol, but slightly soluble in water. The chemical structure of cinnamaldehyde consists of a benzene nucleus substituted by a conjugated carbonyl system. Thisis a feature of organic sunscreen compounds (Ngadiwiyana et al., 2004).

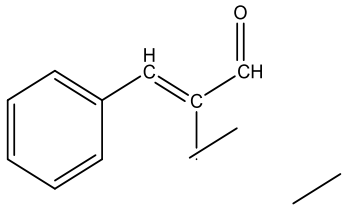

Fig.1. Structure of cinnamaldehyde

So far, it has not been reported about the activity of cinamaldehyde against *Salmonella typhi*. *Salmonella* is a gram-negative, rod-shaped bacterium that is divided into various species. One species of Salmonella that is pathogenic is *Salmonella typhi*. These bacteria can live in human body. Human that infected by *Salmonella typhi* bacteria can excrete these bacteria through respiratory tract secretions, urine, and feces over varying periods of time.

Generally, people that infected by *Salmonella typhi* are fecal-oral, which is *Salmonella typhi* that entered the gastrointestinal tract cannot cause the infection. The infection would occur when *Salmonella typhi* reaches small intestine (Hanna, *et al*, 2005). Infection caused by this bacterium is typhoid fever and can be fatal for the infants, toddlers, pregnant women, and their fetus, also the elders which have un-optimal or dropped immune system.Typhoid fever is transmitted through food and drink that has been contaminated by *Salmonella typhi* or people who have become carriers (people who have had the infection but the bacteria is still present in the body). Symptoms of typhoid fever are fever, nausea, vomiting until cause a death. The pathogenesis of typhoid fever involves 4 processes, ranging from attachment of bacteria to the intestinal lumen, multiplied of bacteria in the Peyer's patch macrophages, surviving in the bloodstream and producing enterotoxins which lead to electrolyte and water discharges to the lumen intestinal.

2. Materials and Methods

2.1. Tools and Materials

Tools that used in this research were: autoclave, petri dishes, incubator, micropipette, inoculation loop, tweezers, aluminum foil, analytical balance, volumetric flask, sterile cotton, gauze, beaker glass, stirring rod, erlenmeyer flask, separating tube, test tube, cylinder, rotary vacuum evaporator, oven, chamber, visible light, UV-Vis and GC-MS spectrophotometer, laminar air flow, and colony counter.

The materials needed in this research are commercial cinnamon oil, sterile water, preparative thin layer chromatography (PTLC), sodium bisulfite, petroleum ether, ethanol, methanol, pure Salmonella typhi, Mueller Hinton Agar medium, amoxicillin, beef broth, Agar powder, and anhydrous Na_2SO_4.

2.2. Methods

2.2.1. Isolation and Characterization of Cinnamaldehyde

Isolation of cinnamaldehyde using bisulfite addition method was carried out by reacting cinnamon oil to the saturated sodium bisulfite solution with a ratio of 1:1. The precipitate formed was further neutralized with 10% HCl solution. Furthermore, cinnamaldehyde was extracted from the solution using petroleum ether twice and washed by sterile water. The petroleum ether extract was applied in the evaporator.

2.2.1. Antibacterial Activity Test

2.2.1.1. Preparation of the Tested Sample

10 mg of the isolated compound dissolved in 100 mL water to obtain the concentration of 100 ppm (as the main liquor). The tested solutions were prepared to be 20 ppm, 40 ppm, 60 ppm, and 80 ppm from the main liquor

2.2.1.2. Preparation of Medium

a. Nutrient agar medium

350 mL of sterile water, 350 mL of brief broth and 20 gram of agar powder were heated until thickened in a beaker glass while stirring.

b. Mueller hinton agar medium

500 mL of sterile water mixed with 20 grams of instant Mueller Hinton Agar then heated to be thickened and yellow in a beaker glass while stirring.

2.2.1.3. Rejuvenation of Tested Bacteria

The bacteria that used as the tested bacteria were inoculated in a 5 mL Nutrient Agar medium in the test tube using the ose needle, at 37 º C for 24 hours, using a scratch method.

2.2.1.4. Preparation of Comparative Solution

0.1 mg of amoxicillin antibiotic was dissolved adequately in sterile water up to 100 mL to reached a concentration of 0.1 ppm. This solution was used as a positive control.

2.2.1.5. Antibacterial Activity Testing Using Agar Diffusion Method (Sumuran)

Bacterial resistance was seen from the diameter of inhibitory zone. 10 mL of Mueller Hinton Medium poured on to sterile petri dishes and allowed to be freeze as the basic layer. After that, 5 mL of the rather cold Mueller Hinton Agar Medium with temperature 45-48 °C mixed well with bacteria up to 6 mL and homogenized. Then poured over the base layer of the medium and distributed evenly using a sterile speader (pour plate method). Furthermore, the incisions were placed on the surface of the medium and filled with 0.2 mL of comparative solution and test solution. A medium consist of 7 tube that divided into a positive control tube which contain amoxicillin, 5 tube which contain difference concentration of methanol extract of cinnamaldehyde and a negative control tube which only contain of sterile water. The incisions were incubated at temperature of 37 ° C for 24 hours. Then the diameter of inhibitory zone or the space that not overgrown by bacterial calculated by using colony counter

3. Results and Discussion

3.1. Isolation and Characterization of Isolates

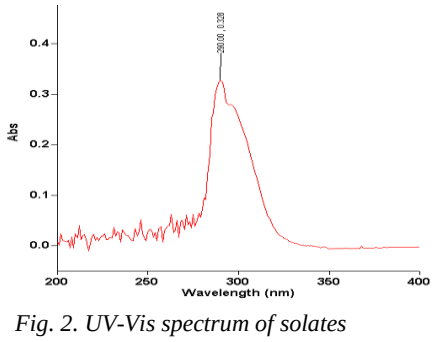

Fig. 2. UV-Vis spectrum of solates

The isolation of cinnamaldehyde using bisulfite addition method obtained a yellow liquid with a yield of 86.79 %.The analysis of isolate by using UV-Vis Spectrophotometer UV-Vis showed a strong absorption at a wavelength of 290.00 nm with an absorbance of 0.328. This wavelength corresponds to the maximum wavelength of cinnamaldehyde (Fig. 2).

Identification of cinnamaldehyde using GC-MS was performed to determine the purity of the isolated cinnamaldehyde by PLTC method. The result revealed that cinnamaldehyde had a purity of 88.41 %.

The analysis using Mass Spectrophotometer gave a molecular weight of 132 with the base peak of 131m/z which resulted by atomic radical of H released, whereas the peak of 103 resulted by the released ofaldehyde's C=O group. All of fragmentations given were identical to cinnamaldehyde's structure. The result of mass spectrophotometer analysis shown in Fig.3.

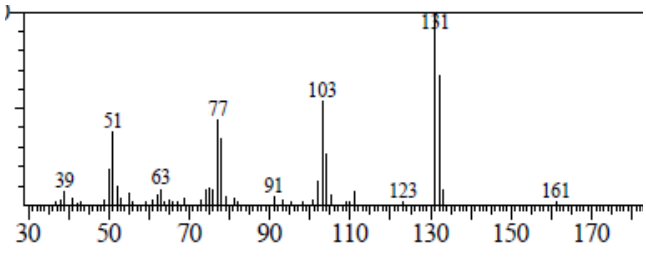

Fig. 3. Mass spectrum of isolates

3.2. Anti Bacterial

The method used in antibacterial activity test of cinnamaldehyde was agar diffusion. In this method, the tested bacteria was bred in medium growth of bacteria then into each medium included various concentration tubes of cinnamaldehyde solution which were 20 ppm, 40 ppm, 60 ppm, and 100 ppm.

Result of antibacterial activity test of cinnamaldehyde that isolated from the cinnamon oil against the growth of *Salmonella typhi* listed in Table 1.

Table 1. The inhibitory zone diameter of cinnamaldehyde against Salmonella typhi

No	Treatment	Diameter of Inhibitory Zone (mm)			Total	Average
		I	II	III		
1	20 ppm	10.4	13.7	11.5	35.6	11.86
2	40 ppm	11.5	14.4	12.6	38.5	12.83
3	60 ppm	12.2	15.2	13.0	40.4	13.46
4	80 ppm	12.6	16.3	13.7	42.6	14.2
5	100 ppm	14.4	17.0	14.8	46.2	15.4
6	K^+	18.2	17.0	14.8	50	16.6
7	K^-	-	-	-	-	-

Captipon : K^+ is a positive control and K^- is a negative control, the data reported in Table 1 showed that the cinnamaldehyde solution has an inhibitory effect on the growth of *Salmonella typhi* as indicated by the mean diameter of different inhibitory zones in each treatment. The increased concentration was in line with the increased of inhibitory zone diameter. According to Davis and Stout (1971), if diameters of the inhibitory area is 5 mm or less, the inhibiting activity is categorized as weak, 6-10 is categorized as moderate, 11-19 mm is categorized as strong, and 20 mm or more is categorized as very strong. This research revealed that cinnamaldehyde

provided a strong inhibitory activity because it had inhibitory zone diameter between 11 and 19 mm at concentration of 100 ppm. Amoxicillin was used as a comparative solution because amoxicillin proved 80% resistance to *Escherichia coli*. Based to the result shown in the Table 1, inhibitory zone diameter of amoxicillin was 16.6 mm. In other word theinhibitory zone diameter of amoxicillinwas more than inhibitory zone diameter of cinnamaldehyde. This was due to the poor concentration of the used cinnamaldehyde. The higher concentration caused the greater released of antimicrobials, thus facilitating the penetration of the compound into the cell. But the concentration of 100 ppm of cinnamaldehyde solution can be quite good in inhibiting bacterial growth compared to the exciting studies. In 2007, Shan declared on his research that at concentration of 28% methanol extract of cinnamon's bark inhibited the growth of *Eschericia coli* with the inhibitory zone diameter of 8.7 mm,and in 2013, Angelica revealed that ethanol extract of cinnamon's leaf in 500.0 ppm of concentration inhibited the growth of *Eschericia coli* with the inhibitory zone diameter of9.0 mm. Then the result of this research was better than the exciting studies because cinnamaldehyde solution of this research provided a considerable inhibitory than the tested solution of the exciting studies. (Shan., et al., 2007) also stated that a tested solution provided a potential as an antibacterial if it had the standard inhibition of 14 mm. According to the statement, can be concluded that concentration of 100 pm cinnamaldehyde had the potential as an antibacterial with inhibitory of 15.4 against *Salmonella typhi*

4. Conclusion

Cinnamaldehyde compounds can be isolated from cinnamon oil (*Cassia oil*) by using by using bisulfite addition method with the yield is 86.79 %. Gas Chromatograph – Mass Spectrophotometer characterization denoted the cinnamaldehy de's molecular weight of 132. Tested solution of Cinnamaldehyde with the concentration of 100 ppm provided the potential as an anti-bacterial in inhibitory zone diameter of 15.40 mm against *Salmonella typhi*, whereas amoxicillin as the compared solution was 16.60 ppm.

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Conflict of interest: Non declare