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Antimicrobial Potential of Miana Leaf Extract (*Coleus scutellaroides*(L) Benth) Against *Salmonella typhi*: In Vitro Study

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Abstract

Introduction*: Salmonella typhi* is a bacilli gram-negative bacteria, belonging to the family *Enterobacteriaceae*, which causes typhoid fever. The high morbidity and mortality rates of typhoid fever and the problem of antibiotics resistance have encouraged development of the use of effective medicinal plants to treat typhoid fever. One of plant widely used as a medicinal plant is miana (*Coleus scutellariodes* (L.) Benth).Miana leaves contains flavonoid and tannin, in which flavonoid has antibacterial activity because it can denature and coagulate bacterial cell protein. **Methods**: Miana extracts were diluted in five concentration: 5%, 25%, 50%, 75% and 90%. Pure culture of *S. typhi* was identified by several biochemical test: TSIA, sulfide production, Indole, motility, vogesproskauer, methyl red and citrate. Inhibition test of extracts against bacteria using the Kirby-Bauer method with blank discs soaked in miana extract solutions, empirical antibiotics and negative control. **Results**: Biochemical tests of *S. typhi* were TSIA: alkali/acid, sulfide production: positive, indole: negative, motility: positive, methyl red: positif, VogesProskauer: negative, and citrate: negative; they were suitable with characteristic of *S. typhi*. Miana extract inhibited the growth of *S. typhi* on concentrations of 5%, 25%, 50%, 75% and 90% with the mean inhibition zone diameter of 1.83 mm, 4.33 mm, 5.00 mm, 5.66 mm, and 6.00 mm, respectively and the average inhibition zone of empirical antibiotic levoflocaxin, chloramphenicol and amoxicillin were 24.17 mm, 21.50 mm and 0.00 mm, respectively.

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Conclusions: Based on the research results obtained, it can concluded that the miana extract were in the moderate categories as an antibacterial base ingredient. Miana extracts on all concentrations were more potent than amoxicillin.

Keywords: Miana; Salmonella typhi; inhibition zone; empirical antibiotic.

1. Introduction

Typhoid fever is an acute infectious disease of the digestive tract which is endemic in Indonesia and transmitted oro-fecally [1-3]. Data from WHO (World Health Organization) estimates that the worldwide incidence rate is around 17 million per year with 600,000 people dying from typhoid fever and 70% of the deaths occurring in Asia (WHO, 2008 in the Indonesian Ministry of Health, 2013) [4,5]. Based on the Indonesian Health Profile in 2010, 41,081 cases of typhoid and paratyphoid fever were hospitalized and 279 of them died (Depkes RI, 2010) [5]. Based on research conducted by MochammadHatta, it was found that the level of antibiotic resistance to *S. typhi* from South Sulawesi, Indonesia was very low (<1%) before 2001 and chloramphenicol remains the treatment of choice. However, since 2001 resistance has increased and in 2007, 6.8% of isolates were resistant to the three first-line drugs: ampicillin, chloramphenicol, and cotrimoxazole. Ciprofloxacin resistance in 2007 was 3.90%.[5] The high morbidity and mortality rates of typhoid fever and the problem of resistance to several antibiotics in the treatment of typhoid fever have encourages the development of a new paradigm in the health sector, it is the use of natural herbs and traditional medicines, which provide opportunities to research and develop effective medicinal plants to treat typhoid fever [6]. One of plant widely used as a medicinal plant is Miana (*Coleus scutellaroides* (L.) Benth).Miana is one of the plants included in the biopharmaceutical plant commodity based on the Decree of the Minister of Agriculture Number: 511/Kpts/PD.310/9/2006 [7]. Miana leaves contain flavonoid and tannin compounds, where flavonoid compounds act as antibacterial because they can denaturate and coagulate bacterial cell protein [8-10].

2. Materials and Methods

2.1. Salmonella typhi bacteria

S. typhi bacteria was from the Biomolecular and Immunology Laboratory, Faculty of Medicine, University of Hasanuddin.

2.2. Miana Leaf Extract

The leaves of miana were obtained from TanaToraja Regency and then extracted at the Fitofarmaka Laboratory of the Faculty of Pharmacy, Hasanuddin University.

2.3. Empirical Antibiotics

The empirical antibiotics referred to antibiotics generally used in the treatment of patients suspected of typhoid fever, they are amoxicillin, chloramphenicol, cotrimoxazole, ciprofloxacin, levofloxacin, and ceftriaxone

injection [11]. The empirical antibiotics used in this study were amoxicillin, chloramphenicol and levofloxacin.

2.4. Culture Media

The culture medium used in this study was the MacConkay agar which was used to culture *S. typhi* bacteria. Then, MHA (Muller Hinton Agar) agar was used as the medium for disk diffusion test (Kirby-Bauer method) [12].

2.5. Miana Extraction

In the first stage, the miana leaves extraction were carried out using four solvents with different polarity. Sample preparation began with leaves selection. The samples were tested qualitatively for phytochemicals. The leaves were weighed with a certain weight and then extracted using four solvents, they were water, ethanol, chlorophromes, and hexane using the immersion method for 3 x 24 hours, filtering every day until a clear filtrate was obtained. The filtrate obtained was concentrated using a rotary evaporator to obtain miana extract. Then, miana extract were diluted in different concentration of 5%, 25%, 50%, 75% and 90% [9].

2.6. Identification of S. typhi

Pure culture of *S. typhi* was identified by several biochemical test: TSIA, Indole, VogesProskauer, Methyl Red and Citrate [13].

2.7. Disk Diffusion test

Disk diffusion test test was carried out to determine the antibacterial effect of miana extract. This test procedures in this study included [14].

1. Streak the *S. typhi* bacteria culture on the Muller Hinton Agar (MHA) plate using a sterile cotton swab
2. Soak the blank discs in the solution of miana leaves at different concentration, 5%, 25%, 50%, 75% and 90%, then air dried for 30 minutes at room temperature in a laminar air flow to remove the solvent.
3. Put the discs on to MHA, included negative control and the empirical antibiotics (amoxicillin, chloramphenicol and levofloxacin)
4. Incubate the media at 37◦C for 24 hours.
5. Measure the zone of inhibition that forms around the disc using a ruler.

2.8. Data Analysis

Inhibition of miana extracts marked by the clear zone in around paper discs. Clear zone around paper disc is the diffusion area of miana extract that affects the growth of bacteria. The diameter of inhibition zone show the

strength of antibacterial of the extract used. Davis and Stout (1971) had classified the antibacterial ability by diameter which was obtained. Extract by inhibition diameter more than 20 mm is included in very strong category, inhibition diameter range from 10-20 mm included in strong category, inhibition diameter ranges from 5-10 mm belongs to medium category and inhibition diameter less than 5 mm included in weak category [14,15].

Diameter of inhibition zone were presented in table. Effectiveness of antibacterial concentrations were compared with antibiotics (levofloxacin) calculated based on the equation (Tangapo, 2005) [14]:

$E = (D / Da) \times 100\%$

E: antibacterial effectiveness (%)

D: diameter of the extract inhibition zone (mm)

Da: diameter of antibiotic inhibition zone (mm)

3. Results and Discussion

Inhibition zones of miana extract to *S. typhi* were formed at all concentration with the smallest zones in concentration 5% (Figure 1). This indicates that the miana extract at low concentrations able to interfere the metabolism of bacteria. Inhibition zone of miana extract progressively raised with increasing extract concentrations.

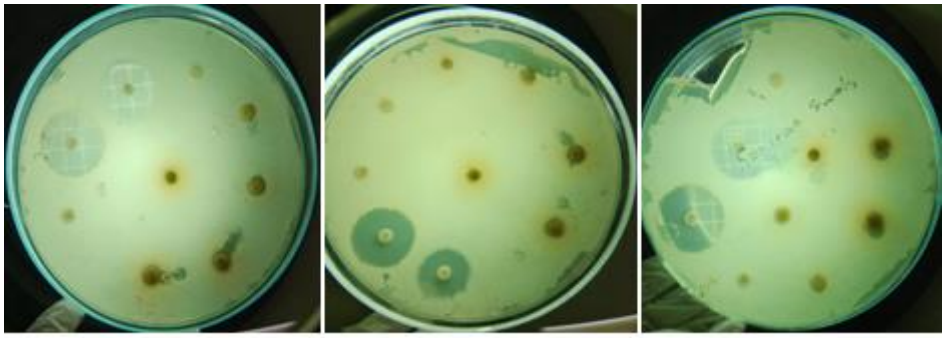

Figure 1: Inhibition zones of miana extract at concentration 5%, 25%, 50%, 75% and 90%; empirical antibiotic: levofloxacin, chloramphenicol, amoxicillin and negative control with three times repetition.

Inhibition of miana extract against *S. typhi* found at all concentration, the lowest inhibition at 5% concentration and the highest at 90% (Table 1). Miana extract with a concentration of 5% and 25% had antibacterial power included weak category because it had a inhibition zone less than 5 mm. Whereas, concentration of 50%, 75% and 90% were included in medium category with inhibition zone ranges from 5-10 mm.

Table 1: The mean diameter of inhibition zone of miana extract against *S. typhi*

Mean diameter of inhibition zone (mm)								
Miana Extract					Empirical Antibiotics			Neg Control
5%	25%	50%	75%	90%	L	C	A	
1.83	4.33	5.00	5.66	6.00	24.17	21.50	0.00	0.00

Abbreviations: L: Levofloxacin; C: Chloramphenicol; A: Amoxicillin

This matter indicated that miana extract has a mechanism of action as an antibacterial and is able to disrupt metabolism bacteria. For empirical antibiotics, levofloxacin had the highest inhibition zone (mean diameter 24,17 mm) and the lowest was amoxicillin (mean diameter 0.00 mm). It showed that miana extract were more potent than amoxicillin as empirical therapy. Miana leaves contains flavonoid and tannin, in which flavonoid has antibacterial activity because it can denature and coagulate bacterial cell protein [8]. Recent study showed that miana extract contains active substances such as alkaloids, saponins, steroids, tannins, potential as immunomodulators [16]. Other study stated that the main active substance of the plant *Coleus aromaticus* is an essential oil. Essential oils have a substantial antimicrobial activity in gram negative, gram-positive, drug-resistant microorganisms, phytopathogenic microorganisms and fungi [16-17]

Table 2: Antibacterial Effectiveness of Miana Extract

Concentration (%)	Effectiveness (%)
5%	7.57
25%	17.91
50%	20.68
75%	23.42
90%	24.82

Table 3 showed that miana extract 5% had the lowest antibacterial effectiveness against *S.typhi* (7.57%). Low effectiveness was due to limit concentration of active compounds in the extract. Otherwise, miana extract 90% had the highest antibacterial effectiveness (24.82%). This was due to concentration of active compounds in extract used was more than others. The limitation of this study was the use of crude extracts. The utilization of extracts with single active compounds which has been known as an antibacterial will be more effective in inhibiting bacterial growth.

4. Conclusion

Based on the research results obtained, it can concluded that the miana extract were in the moderate categories as an antibacterial base ingredient. Miana extracts on all concentrations were more potent than amoxicillin.

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5. Competing Interest

The authors declare that they have no competing interests

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