Ethanolic extract of local sorghum seedcoat (*Sorghum bicolor* [L.] *Moench*) as a potential bacterial growth inhibitor

N I Inda¹, S Mastura¹ and P Satrimafitrah¹*

¹Department of Chemistry, Tadulako University Jl. Soekarno Hatta Km.9, Kampus Bumi Tadulako Tondo Palu,

*E-mail: pasjan82@gmail.com

Abstract. Research on the antibacterial activity of ethanol extract from seedcoat of local Sorghum (*Sorghum bicolor* [L.] *Moench*) against *Staphylococcus aureus* and *Escherichia coli* has been conducted. The purpose of this study was to determine the minimum inhibitory concentration of extract against both gram-positive and negative bacteria. The extraction method used in this study was a maceration method using ethanol as a solvent. Antibacterial activity test was carried out with several concentrations, namely 1 g/mL, 0.8 g/mL, 0.6 g/mL, 0.4 g/mL, 0.2 g/mL, 0.1 g/mL, 0.05 g/mL, 0.025 g/mL, and 0.01 g/mL. The results showed that the extract inhibited bacterial growth in a wide range of concentrations. The smallest inhibition was at a concentration of 0.05 g/mL with a clear zone diameter of 10.29 mm in *Staphylococcus aureus* and 11.70 mm in *Escherichia coli*. Ethanol extract of local Sorghum seedcoat exhibited a strong inhibition ability and probably a potential antibacterial agent source.

1. Introduction

About 80% of developing countries' population still rely on traditional medicine, and 85% of conventional medicine in practice uses plants [1]. Indonesia is known to be rich in medicinal plants; some medicinal plant species have been used as traditional medicine. The Indonesian people have widely recognized the use of natural ingredients as traditional medicine. It can be seen from the number of traditional herbal products, both processed with modern and straightforward techniques. The use of plants that are used as a medicine, in general, is to prevent various kinds of diseases, to maintain and restore health [2].

People have generally used ingredients from fresh or dried medicinal plants that are stored for later use, which is called simplicia [3]. Some plant extracts can inhibit bacterial growth and are antimicrobial. Plants are brimful with a wide selection of secondary metabolites, like alkaloids, flavonoids, terpenoids, and tannins with antimicrobial properties [4]. Plants such as *Artemisia vulgaris* L and *Sorghum bicolor* *L. Moench* tested positive as antimicrobial agents and contained flavonoids, saponin, and tannin [5,6].

Sorghum is a prospective alternative commodity in foods, feeds, and industries in Indonesia [7]. Sorghum, a cereal type of food, is a potential commodity source of carbohydrates. However, the main problem in using sorghum seeds as food and feed is relatively high tannins, reaching 0.40–3.60% [8]. The bitter taste from tannins causes a bad and dry feeling in the mouth following the consumption of tea, unripened fruit, or any seed containing tannin [9]. Thus, people will tend to discard or separate seedcoat from sorghum seeds before processing it further and leaving seedcoat as waste material. To date, the information related to the antibacterial activity of the seedcoat of sorghum is little. In this work,
we present ethanolic extracts of seedcoats from local Sorghum bicolor [L.] Moench, a potential antibacterial agent for both gram-positive and gram-negative bacteria.

2. Experimental procedures

2.1. Sample preparation
The local sorghum seeds were dried, powdered until seeds and coats separated. Seed coat then collected, mashed, and sieved into 60 mesh. Next, meshed seed coats were stored for later use.

2.2. Extraction of seedcoat
Extraction was carried out using the maceration method with ethanol solvent. One hundred grams of powdered sorghum seedcoat weighed then put into 1000 mL Erlenmeyer. Furthermore, 500 mL of ethanol was added. The mixture is stirred for 10 minutes, after which it is stored for 3 x 24 hours, then filtered by vacuum. Following the evaporation process, the obtained extract was then stored for later use.

2.3. Antibacterial activity test
Antibacterial activity was performed using the agar disk diffusion method [10]. Nutrient agar (NA) media was mixed with the bacterial suspension of *Staphylococcus aureus* or *Escherichia coli*, homogenized, then poured into a sterile petri dish and allowed to solidify. After that, wells with a diameter of ± 9 mm were prepared. Each plate contains three holes or wells (the first hole for the negative control, the second hole for positive control, and the third hole for the sorghum seed coat extract with a concentration of 1 g/mL). Other plates with concentration ranging from 0.8 g/mL to 0.01 g/mL were prepared as well. Next, the plates were incubated for 24 h at 30 °C and the inhibition zone was measured. The experiments were done in triplicates.

2.4. Optical Density (OD) measurement
Bacterial growth was calculated using turbidity measurement with some modification [11]. Two bacterial cultures of *Staphylococcus aureus* and *Escherichia coli* were prepared and incubated for 24 h at 37 °C and 180 rpm in Lysogeny Broth (LB) medium. As much as 100 μL of the extract was added, then incubated further for 48 h. Bacterial cultured in LB medium without extracts or with antibiotics were prepared as the negative and positive controls. The absorbance was measured using a UV-Vis spectrophotometer at OD of 600 nm every 0 hours, 3 hours, 6 hours, 9 hours, 12 hours, and 24 hours. The experiments were performed with three repetitions.

2.5. Statistical analyses
One way ANOVA was applied to analyze the difference between various concentrations of extracts and bacterial inhibition. P < 0.05 was used to conclude statistical significance.

3. Results and discussion
Sorghum seed coat simplicia powder was immersed in 500 mL of 96% ethanol for three days while stirring 1-2 times every day to accelerate the contact between the solvent and the simplicia. The maceration results are then filtered, then concentrated using a rotary vacuum evaporator with a 45 °C temperature for 1 hour to separate the active substance and the solvent. Next, we tested the antibacterial activity from the obtained ethanolic extract.

Antibacterial activity tests against *Staphylococcus aureus* and *Escherichia coli* at a concentration of 0.8 g/mL, 0.6 g/mL, 0.4 g/mL, 0.2 g/mL, 0.1 g/mL, 0.05 g/mL, 0.025 g/mL, and 0.01 g/mL showed differences in the inhibition zone. The antibacterial activity test was carried out by the diffusion well method, which was characterized by the formation of a clear zone around the well, positive control in the form of chloramphenicol, while negative control was in the form of DMSO. Observation of the diameter of the clear zone was measured using a caliper.
As seen in Table. 1, the ethanol extract at a concentration ranging from 1 g/mL to 0.6 g/mL indicated comparable inhibitory capability against both *S. aureus* and *E. coli*. The concentration ranging from 0.4 g/mL to 0.1 g/mL, exhibiting the slight difference in inhibition strength as extracts, gave a more substantial effect on *E. coli*, a gram-negative bacteria. Next, to determine the differences in ethanolic extract concentrations given to *S. aureus* and *E. coli* bacteria growth, we tested the data using One Way Anova. The results obtained p-value 0.0009 < α=0.05, concluding that addressing different concentrations of ethanolic extracts of sorghum seedcoat will affect bacterial growth.

**Table 1. Antibacterial activity of ethanolic extracts from sorghum seedcoat with varied concentration**

| concentration  | clear zone (mm) |
|---------------|-----------------|
|               | *S. aureus*     | *E. coli*     |
| 1 g/mL        | 18.49           | 18.67         |
| 0.8 g/mL      | 18.33           | 17.84         |
| 0.6 g/mL      | 17.60           | 17.27         |
| 0.4 g/mL      | 15.43           | 17.08         |
| 0.2 g/mL      | 13.91           | 15.80         |
| 0.1 g/mL      | 11.47           | 14.36         |
| 0.05 g/mL     | 10.29           | 11.70         |
| 0.025 g/mL    | 0               | 0             |
| 0.01 g/mL     | 0               | 0             |
| Chloramphenicol (0.01g/mL) | 27.36 | 30.56 |
| DMSO          | 0               | 0             |

At the two lowest concentrations (Table. 1), the ethanolic extracts of sorghum seedcoat did not inhibit the growth of both bacteria. The diameter of a clear zone is influenced by several things, such as the sensitivity level of the test organism, the diffusion rate of antibacterial compounds, and the concentration of antibacterial extracts or compounds [12]. The antimicrobial activity from methanol, n-hexane, and butanol extracts of total crude *Sorghum bicolor* was reported [13]. Recently, its antibacterial activity from leaves has been reported as well [14]. Thus, our work is the first to report about antibacterial activity from the seedcoat.

![Figure 1](image1.png) **Figure 1.** *S. aureus* growth curves with different concentrations of seedcoat extracts (average ± standard deviation, n = 3)

![Figure 2](image2.png) **Figure 2.** *E. coli* growth curves with different concentrations of seedcoat extracts (average ± standard deviation, n = 3)

Interestingly, when we tested the effect of the two lowest ethanolic extracts of seedcoat against both bacteria by using the OD₆₀₀ method, we observed a slight delay of bacterial growth (Figure 1 and Figure 2). At a concentration of 0.4 g/mL, bacterial growths were delayed up to 47 % (*S. aureus*) and 50 %
(E.coli), suggesting it inhibited gram-negative bacteria stronger. Chloramphenicol (0.01 g/mL) killed bacteria as observed from OD$_{600}$ at 3 – 24 h incubation (data not shown). OD$_{600}$ method is equivalent to the total bacterial number even though it does not present its viability. While disk diffusion, it represents viable yet dead bacteria that remain undetected. Disk diffusion limitations depend on the solubility of extracts and diffusions to the surrounding agar media, while the OD$_{600}$ enables monitoring and measuring bacterial growth repeatedly [15,16].

Previous work reported that leaves of S. bicolor contained alkaloids, flavonoids, steroids, saponins, and tannins [14]. It is not only phenolic compounds present in plant extracts that inhibit bacterial growth but also various secondary metabolites [17]. It was reported that tannic acids from the whole sorghum had a high level against E.coli [18]. Therefore, the inhibition capability of our sorghum seedcoat extracts probably comes from tannin as well.

4. Conclusion

Our present work suggested that ethanolic extracts from local Sorghum bicolor [L.] Moench seedcoat has potential antibacterial activity, with a more substantial effect on gram-negative bacteria. It is suggested to isolate its secondary metabolite compounds for further study.

Acknowledgements

The authors would like to express special gratitude to Dewi for her technical assistance.

References

[1] Gana A K 2008 African Journal of General Agriculture 4(1)
[2] Yusuf L H 2017 J Plant Pathol Microbiol 8:5
[3] Syahrurachman A et al 1994 Mikrobiologi Kedokteran Edisi Revisi (Jakarta: Binarupa Aksara) pp 163-165.
[4] Cowan M M 1999 Clinical microbiology reviews 12(4) 564–582
[5] Febrina L et al 2017 Jurnal Pendidikan Kimia 9(2) 311-317
[6] Awika J M and Rooney LW 2004 J. Phytochem. 65 1199-1221
[7] Sirappa M P 2003 Jurnal Litbang Pertanian 22(4)
[8] Rooney L W and Sullines R D 1977 The Structure of Sorghum and Its Relation to Processing and Nutritional Value (Texas: Cereal Quality Laboratory Texas University) pp 91–109
[9] McGee H 2004 On food and cooking: the science and lore of the kitchen (New York: Scribner) p 714
[10] Heatley N G 1944 Biochem. J 38 61-65
[11] Harnack K, Spolaczyk R and Janke S A 1999 Biospektrum 1999 6 503-504
[12] Prescott L M 2005 Microbiology (Iowa: William C. Brown Publisher) pp 415-476
[13] Young K H, Soo SE and Kumar G B, et al 2009 Food Chemistry 115(4) 1234-1239
[14] Elkhatim et al 2020 GSC Biological and Pharmaceutical Sciences 10(01) 065-072
[15] Haase H, Jordan L, Keitel L, Keil C and Mahltig B 2017 PLOS ONE 12(11)
[16] Bauer A W, Kirby W M, Sherris JC, and Turck M 1966 Am J Clin Pathol 45 493–496
[17] Gordana S C et al 2007 International Journal of Molecular Sciences 8 1013–1027
[18] Lee J S et al 1994 Korea Journal of Nutrition 27 819–827