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Antibacterial Activity of Ethanol Extract of Arabian Bidara Leaves *(Ziziphus spina-christi L)* and Bioautography TLC Analysis Against Pathogenic Bacteria

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Abstract

The Ziziphus spina-christi L. plant, commonly known as Arabian bidara, are rich in potentially health-beneficial compounds, particularly those with antibacterial properties. This research aimed to investigate the effectiveness of an ethanolic leaf extract in inhibiting the growth of pathogenic bacteria. The leaves were subjected to maceration using 70% ethanol, followed by an assessment of their antibacterial properties using the agar diffusion method. Subsequently, the active extract was analyzed through bioautography using thin-layer chromatography (TLC), and its phytochemical spotted was screened. The results of the antibacterial activity using the ethanolic leaf extract revealed that it exhibited antibacterial activity when applied at a concentration of 2 mg per paper disk. Subsequently, it showed inhibition zones with diameters of 19.44 mm for Bacillus subtillis; Escherichia coli (17.06 mm); Staphylococcus aureus (16.32 mm); Pseudomonas aeruginosa (12.96 mm), and Salmonella typhi (14.50 mm). The TLC-bioautography analysis of the ethanolic leaf extract demonstrated inhibitory activity against Bacillus subtillis of Rf value with 0.96, Escherichia coli (0.31) and Staphylococcus aureus (0.89). However, no inhibitory activity was observed against Salmonella typhi. The phytochemical screening results suggested the presence of flavonoids, terpenoids, and tannins in the ethanolic leaf extract.

Keywords: Antibacterial, Bidara arab leaves *(Ziziphus spina-christi L)*, TLC-Bioautography..

INTRODUCTION

Indonesia possesses a rich and diverse array of plant species that serve as valuable sources of raw materials for traditional medicinal practices, comprising both standardized consumption and empirically derived recipes (Yuslianti et al., 2016). Despite the extensive reliance on medicinal plants within Indonesia, the utilization of these raw materials remains suboptimal, as the country still relies on imports from foreign sources, primarily China and India (Arista et al., 2022). These plants are of significant importance due to their potential to yield essential medicinal components for combatting infectious diseases, which constitute an ongoing challenge in the continually evolving field of healthcare. These infections may caused from various microorganisms including bacteria, viruses, fungi, and protozoa. As such, antibiotics are pivotal in the realm of anti-infection treatments. Nonetheless, it is crucial to acknowledge that the persistent and indiscriminate use of antibiotics can lead to the development of antibiotic resistance, thereby rendering certain antibiotics ineffective in therapy (Negara, 2014). Consequently, a number of empirical treatments employing traditional medicines that have been

passed down through generations have emerged as a viable option for the local community in the management of infections.

Arabic leaves, a commonly employed traditional remedy, are indicative of the enduring trust of the general populace in traditional medicine (Siregar, 2020). Abalaka's et al., (2010) research further attests to the therapeutic potential of leaf extracts in the management of infections, including wound infections, meningitis, and infections caused by a variety of microorganisms. Examination of secondary metabolite compounds in leaves revealed the presence of alkaloids, saponin, steroids, triterpenoid, and tannin (Safrudin & Nurfitasari, 2018). The primary objective of this study was to assess the antibacterial efficacy of ethanol extracts derived from leaves in impeding the proliferation of pathogenic bacteria through the utilization of TLC Bio-autography analysis.

RESEARCH METHODS

Sampling, Preparation and Extraction Process

Plant materials were collected from Takalar Regency South Sulawesi, Indonesia. The collected plants were watery washed, disinfected, rinsed with distilled water and finally dried in shade. Subsequently, the leaves were coarsely ground and passed through a No. 18 sieve. For the maceration process, 100 grams of simplicia were weighed and placed in a maceration vessel. Then, a wetting solution comprising 70% ethanol, twice the weight of the sample, was added. The maceration procedure was allowed to continue for a period of 3-4 days, after which the solvent was filtered and subjected to evaporation using a rotary evaporator operating at a temperature range of 50-60° C and a rotational speed of 80-150 rpm.

Antibacterial Activity Test and TLC-Bioautography.

The concentrated extract was diluted to a 10% concentration using DMSO as the solvent. Subsequently, a mixture of 10 mL of NA medium and 20 µL of the test bacterial suspension was prepared in a bottle and then poured onto a Petri dish. A paper disc loaded with 20 µL of the 10% leaf ethanol extract was placed on the medium. Gentamicin served as the positive control, while DMSO was used as the negative control. The Petri dishes were then incubated for 24 hours, and the diameter of the inhibition zones was recorded.

In the following step, TLC-Bioautography was conducted by combining 10 mL of MHA medium with 0.2 µL of the test bacterial suspension and pouring this mixture into a Petri dish. The eluted chromatography plate was positioned on the surface of the medium and stored in a refrigerator for 2 hours. After the designated time, the plate was removed, and the Petri dish was incubated for 24 hours at 37° C. Subsequently, the plate was examined for spots that exhibited inhibitory effects on the growth of the test bacteria based on their Rf values.

Phytochemical Screening

The TLC plates were heated in an oven at 100° C for 30 minutes. The Arabic bidara leaf extract was spotted on a TLC plate and left for a few minutes to dry, then put into a chamber containing ethanol eluent: ethyl acetate (1:3). The spots that had been eluted were observed under UV light with wavelengths of 254 nm and 366 nm. After that, the eluted plate was sprayed with Dragendorf, Lieberman-Burchard, cytoroborate and iron (III) chloride reagents to detect compounds in the extract by looking at changes in the color of the stain

RESULTS AND DISCUSSION

The leaves of the Arabic bidara plant were determined at the Makassar State University Biology Laboratory with certification number 6/UN36.1.4/BIO/SKAP/2020 stating that the plant comes from the kingdom Plantae, division Magnoliophyta, class Magnoliopsida, order Rhamnales, family

Rhamnaceae, genus Ziziphus , and the species Ziziphus spina-christi (L.) Desf. The key to determining the plant is 1b - 2b - 3b - 4b - 6b - 7b - 9b - 10b - 11b - 12b - 13b - 14a - 15a - 109b - 119b - 120b - 128b - 129b - 135b - 136b - 139b - 140b - 142b - 143b - 146a - 147b - 150b - 151a - Fam.Rhamnaceae.

The ethanol extract of leaves which was obtained from the maceration process with 70% ethanol solvent was 6.03 grams with a yield percentage of 6.03%. Ethanol was used as a solvent in this research because it is known that ethanol solvent is polar and can penetrate cell wall materials, resulting in cell diffusion which causes the release of bioactive compounds and is often also used as a preliminary extraction (Yulianti et al., 2021). The antibacterial activity of the ethanol extract was carried out using the diffusion method. Antibacterial activity was indicated by the presence of a clear zone around the paper disc containing ethanol extract of arabic bidara leaves (Table 1 and Figure 1).

Table 1. Antibacterial activity of ethanol extract of Arabian bidara leaves

Pathogenic bacteria	Diameter of inhibition zone (mm)
Bacillus subtilis	19.44
Escherichia coli	17.06
Staphylococcus aureus	16.32
Pseudomonas aeruginosa	12.96
Salmonella thyposa	14.50

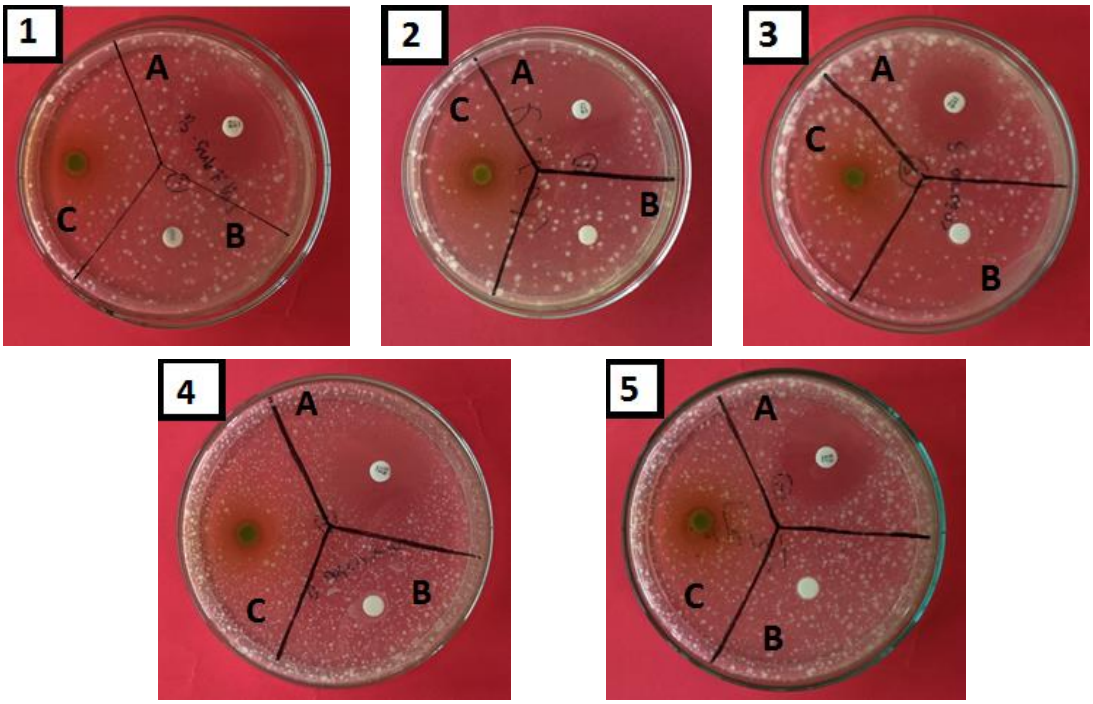

Figure 1. Antibacterial activity of ethanol extract of Arabian bidara leaves (A) positive control gentamicin, (B) negative control DMSO, (C) ethanol extract against *Bacillus subtillis* (1), *Escherichia coli (*2), *Staphylococcus aureus* (3), *Pseudomonas aeruginosa* (4), and *Salmonella typhi*

The test results showed that the ethanol extract leaves was able to inhibit the growth of *B. subtillis*, *E. coli*, *S. aureus*, *P. aeruginosa* and *S. typhi*. The diameter of the inhibition zone resulting from a 10% ethanol extract concentration when tested against the bacteria indicated strong antibacterial activity with a strong inhibitory response category, specifically ranging between 11-20 mm (Purwanto, 2015). The chromatographic profile of the leaf extract, utilizing ethanol eluent in a ratio of ethyl acetate (1:3) with a silica GF_{254} stationary phase, exhibited multiple compound spots. The antibacterial activity associated with these spots will be assessed through TLC-Bioautography.

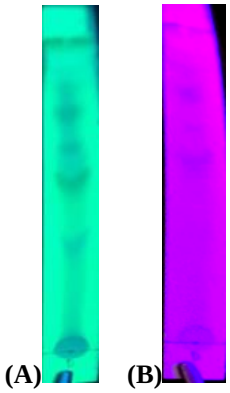

Figure 2. Chromatogram of ethanol extract of Arabian bidara leaves with ethanol eluent: ethyl acetate (1:3). (A) UV254 nm, (B) UV366 nm

The results of TLC-bioautography conducted with the ethanol extract of leaves extracts were revealed a spot exhibiting antibacterial activity against *B. subtillis* bacteria (R_f 0.96), *E. coli* (R_f 0.31), *S. aureus* (R_f 0.89), and *S. typhi* bacteria, while no other spots exhibited inhibitory properties. The appearance of a distinct clear zone on the medium's surface serves as an indicator of the presence of antibacterial compounds.

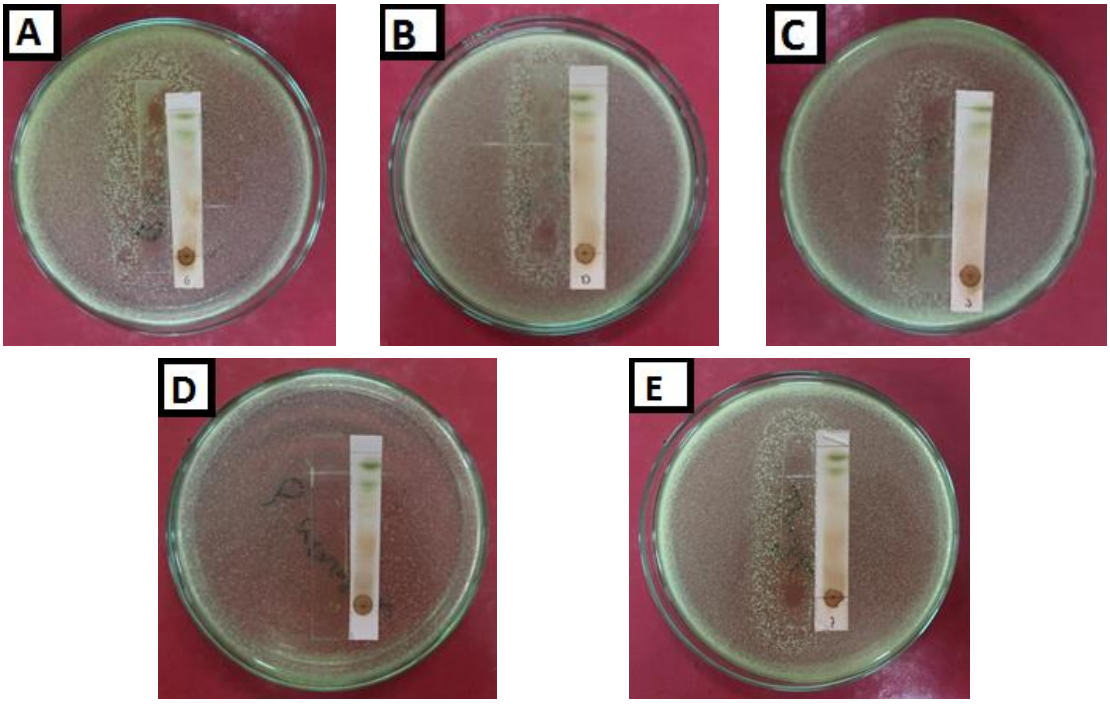

Figure 3. TLC-Bioautography assay of ethanol extract of Arabian bidara leaves against *B. subtillis* (A), *E. coli* (B), *S. aureus* (C), *P. aeruginosa* (D), and *S. typhi* (E)

Phytochemical screening was carried out to determine the group of compounds in the extract that provide antibacterial activity. Phytochemical screening uses Dragendorf, Lieberman-Burchard, citroborate, and iron (III) chloride reagents to identify the compounds contained in the stain so that it can be predicted how compounds from the ethanol extract of arabic bidara leaves will work which are thought to produce antibacterial performance. Based on the findings of the phytochemical screening, it is evident that the active zones observed in the TLC-Bioautography test results of the ethanol extract derived from Arabic bidara leaves against various bacteria contain specific compounds. Subsequently,

the activity of extract against *B. subtillis* (R_f 0.96), these zones contain flavonoids, *E. coli* (Rf 0.31) are terpenoids and *S. aureus* (Rf 0.89) are consist of tannins.

Flavanoids as antibacterial compounds have several mechanisms of action, such as inhibiting cytoplasmic membrane function, inhibiting nucleic acid synthesis, and inhibiting ATP metabolism from bacteria (Manik et al., 2014). Meanwhile, terpenoid compounds work by reacting with the porins of the outer membrane of the bacterial cell wall, then forming strong polymer bonds which will result in damage to the porins so that the bacterial cells will lack nutrition and bacterial growth will be hampered (Cowan, 1999). Tannin compounds exert their antibacterial effects by interacting with polypeptides within the bacterial cell wall, resulting in the disruption of the structural integrity of the bacterial cell wall, ultimately leading to the demise of bacterial cells. Furthermore, tannin compounds possess the capability to deactivate bacterial enzymes and perturb the protein flow within the intracellular layers (Ngajow et al., 2013).

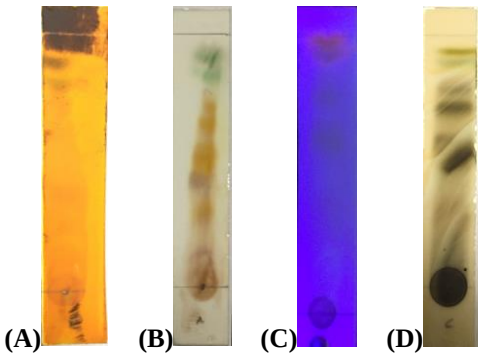

Figure 4. Phytochemical screening chromatogram of ethanol extract of Arabian bidara leaves (A) Dragendorf reagent, (B) Lieberman-Burchard, (C) Citroborat, and (D) Iron (III) chloride).

CONCLUSION

Based on the results of this research, it can be concluded that 70% ethanol extract of Arabian bidara leaves at a concentration of 10% (2 mg/paperdisk) able to inhibit the growth of B. subtillis, E. coli, S. aureus, P. aeruginosa, and S. typhi. The results of the TLC-Bioautography test showed antibacterial activity against the bacteria *B. subtillis*, *E. coli*, *S. aureus* with Rf values of 0.96; 0.31 and 0.89, respectively.

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