ANALYSIS OF TLC-BIOAUTOGRAPHY OF ANTIMICROBIAL COMPOUNDS FROM THE ETHANOL EXTRACT OF LOCAL PALU SHALLOT ROOTS (Allium cepa L. var. Aggregatum) AGAINST Staphylococcus aureus AND Candida albicans

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Shallot locally Palu (Allium cepa L. var. Aggregatum) is a local superior crops that can grow well in dry climates, especially in the Valley of Palu. The content of compounds found in local Palu shallots are include flavonoids, tannins, saponins and alkaloids. Several classes of flavonoids, tannins, saponins and alkaloids have antibacterial and antifungal activity. This study aims are to determine the antimicrobial active compounds from ethanol extract of the roots of local Palu shallot which can inhibit Staphylococcus aureus and Candida albicans using TLC-Bioautography analysis. Determination of the group of active antimicrobial compounds was identified on the TLC plate with the eluent ratio of chloroform:methanol (8:2) and obtained 8 spots which were thought to be the active compound, namely flavonoids (Rf 0,12; 0,18; 0,30; and 0,52), saponins (Rf 0,75 and 0,82), tannins (Rf 0,80) and alkaloids (Rf 0,98). The results of the TLC-contact Bioautografi identified 1 spot stain at Rf 0,98 which can inhibit the growth of Staphylococcus aureus and Candida albicans. The characterization of the spots was carried out by the appearance of H2SO4 10%, Dragendorff and FeCl3 1% spots and it was suspected that the spots that inhibited the growth of the tested microbes were alkaloid compounds.

Keywords: Shallot root, antimicrobial, Staphylococcus aureus, Candida albicans, TLC-bioautography.

INTRODUCTION

Shallots are annual plants from the Liliaceae family that grow almost all over the world. Shallots are a genus of Allium, whose tubers are often used as a spice or flavoring for food and have various medicinal properties. Functioning as benefits and benefits as a kitchen, it turns out that onions are known to have antioxidant and antifungal activity. Central Sulawesi, especially in the Palu Valley, which has a dry climate, there are local superior types of shallot commodities that can grow and reproduce well. This local shallot was developed by farmers in Donggala Regency, so it is commonly referred to as "Palu Shallot". The uniqueness and specific properties of this local shallot is that it remains savory or crunchy, and the aroma does not change even though it is stored for a long time, so this shallot is only used for making fried onions. Several studies that have examined Palu's local shallots show that Palu's local shallots are not only used for making fried onions. However, this local shallot can be used as a medicinal ingredient because of the presence of secondary metabolite compounds that have potential as antimicrobials. Based on previous research, it was reported that the ethanol extract of Palu's local shallot skin (Allium cepa L. var. Aggregatum) contains flavonoids, tannins, alkaloids, saponins and has antibacterial activity against Staphylococcus aureus. This research was also confirmed by Faidah (2019) that the ethanol extract of the local Palu shallot root (Allium cepa L. var. Aggregatum) contains alkaloid compounds, tannins, saponins and flavonoids. According to stated that
secondary metabolites contained in the skin of shallots include alkaloids, flavonoids, terpenoids, saponins, polyphenols, and quercetin which have antimicrobial activity. This previous study showed that there were secondary metabolites that had antimicrobial activity in the ethanol extract of local Palu shallots (Allium cepa L. var. Aggregatum), but it was not known which compounds were thought to have antimicrobial activity.

Therefore, the types of compounds that act as antimicrobials can be identified using the TLC-Bioautography method. Bioautographic methods are important to do in order to determine the direct biological activity of complex compounds, especially those related to the ability of a compound to inhibit microbial growth. Several studies that analyzed secondary metabolite compounds such as flavonoids, tannins, alkaloids that have antimicrobial activity using the TLC-Bioautography method, namely the research of reported an inhibition zone of chayote leaf extract with an Rf value of 0.49 in Porphyromonas gingivalis bacteria and Streptococcus mutans which is a flavonoid compound. The study by reported that alkaloid compounds from Sponges Xestospongia Sp had inhibitory activity against Staphylococcus aureus with an Rf value of 0.37. Research by reported that the components of senggani leaf compounds that act as antimicrobials are included in the phenolic group, namely tannins with an Rf value of 0.94 which is blackish blue after being sprayed with FeCl$_3$ 1%. According to, srikaya leaves in the bioautography method show that terpenoid compounds can inhibit Staphylococcus aureus and Candida albicans bacteria.

Based on the description above, it is necessary to identify secondary metabolites in the ethanol extract sample of the local Palu shallot root (Allium cepa L. var. Aggregatum) which is thought to have an antimicrobial role against Staphylococcus aureus and Candida albicans with TLC-Bioautography analysis to obtain theoretical data and scientific evidence.

MATERIALS AND METHODS

Materials
The materials used in this research are extract of shallot root, ethanol 96%, Staphylococcus aureus, Candida albicans, nutrient agar, Potato Dextrose Agar, Potato Dextrose Borth, physiological NaCl 0.9%, distilled water, Dragendorff reagent, FeCl$_3$ 1%, silica gel plate F$_{254}$, methanol, chloroform, H$_2$SO$_4$ 10%, cotton, HVS paper, aluminum foil, rubbing alcohol, and label paper. The tools used in this research are analytical balance, autoclave, incubator, Laminar Air Flow, oven, UV lamp, shaker, developer vessel, capillary tube, beaker 50 mL, petri dish, erlenmeyer 500 mL, measuring cup 50 mL, tube reaction, hot plate, rubber band, loop needle, dropper pipette, micro pipette, test tube rack, bunsen, spatula, matches, blue tip, tweezers, marker, ruler, hair drayer and calipers.

Methods
Sterilization
The glassware used is sterilized first by using an oven at 160°C for 1 hr and the media is sterilized in autoclave at 121°C for 15 minutes. Media Making
NA Media
As much as 1 g of NA (ready to use) was dissolved in 50 mL of distilled water, heated to boiling and completely dissolved, then sterilized in autoclave at 121°C with a pressure of 1 atm for 15 min.
PDA Media
A much as 1 g of PDA (ready to use) was dissolved in 25 mL of distilled water, heated to boiling and completely dissolved, then sterilized in autoclave at 121°C with a pressure of 1 atm for 15 min.

Microbial Culture Rejuvenation
The method used in microbial inoculation was 1 osees microbe (Staphylococcus aureus ATCC 25923, and Candida albicans ATCC 32769) was taken and scratched on the medium to slant, then incubated at 37°C for 24 hrs.

Preparation of Microbial Suspensions
Take 1-2 microbial osees (Staphylococcus aureus and Candida albicans), then put them in an erlenmeyer containing 10 mL of physiological NaCl 0.9% solution. Then the mixture is shaken until homogeneous.
Separation of compounds by TLC

The separation of compounds by TLC was carried out using a silica gel plate F$_{254}$ as the stationary phase. Each plate with a size of 6x1 cm was prepared. Then, before use, the TLC plate needs to be activated by heating it in an oven for 10 minutes at a temperature of 110°C. Furthermore, the sample of shallot root ethanol extract was dissolved with ethanol 96% and then spotted at a distance of ± 1 cm from the lower edge of the TLC plate using a capillary tube. Then dried and put back on the plate. After that, the plates were eluted using Chloroform : methanol (8:2) eluent to the upper limit of the TLC plate in the chamber. Next, the TLC plate is removed from the chamber and aerated. The dry plate was observed for spot spots that arise using a UV lamp at a wavelength of 254 nm. Identification of the compounds separated from the TLC plate using the Rf value as follows$^{14}:

\[
Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent}}
\]

The eluted TLC plates were identified by using a spray reagent by spraying a universal stain-looking reagent, namely sulfuric acid 10% (saponins, tannins), FeCl$_3$ 1% (flavonoid detection) and Dragendorff reagent (alkaloid detection) on the TLC plate. Positive results for saponins are purple, blackish green for flavonoids, brownish orange for alkaloids, and yellowish green (violet-green-yellow) for tannins$^{15}$.

Antimicrobial Activity Test with TLC Analysis-Contact Bioautography

2 sterile petri dishes were prepared and each filled with 10 mL of NA (bacteria) and PDA (fungus) media that had been inoculated with 100 µL of the suspension of Staphylococcus aureus bacteria and the fungus Candida albicans, then waited to solidify. Furthermore, the TLC plate was removed again and the petri dishes were incubated at 37°C for 24 hrs. Observations were made by looking at the inhibition zone which was formed as a clear area that was not covered by microbes (Staphylococcus aureus and Candida albicans) at 18-24 hrs$^{12}$.

Separation of compounds by TLC

Separation and identification of active compounds from the ethanol extract of local Palu shallot roots was carried out using TLC, UV light and spotting. The mobile phase used is a mixture of chloroform:methanol (8:2). The stationary phase used was the silica gel plate F$_{254}$. The spray reagents used in this study

<table>
<thead>
<tr>
<th>Spots</th>
<th>Value Rf</th>
<th>$\text{H}_2\text{SO}_4$ 10% Reagent</th>
<th>FeCl$_3$ 1% Reagent</th>
<th>Dragendorff Reagent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.12</td>
<td>Green Black</td>
<td>Black</td>
<td>-</td>
<td>+ Flavonoids</td>
</tr>
<tr>
<td>2</td>
<td>0.18</td>
<td>Light Green Black</td>
<td>Black</td>
<td>-</td>
<td>+ Flavonoids</td>
</tr>
<tr>
<td>3</td>
<td>0.30</td>
<td>Light Green Black</td>
<td>Black</td>
<td>-</td>
<td>+ Flavonoids</td>
</tr>
<tr>
<td>4</td>
<td>0.52</td>
<td>Green Black</td>
<td>Black</td>
<td>-</td>
<td>+ Flavonoids</td>
</tr>
<tr>
<td>5</td>
<td>0.62</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not Detected Yet</td>
</tr>
<tr>
<td>6</td>
<td>0.70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not Detected Yet</td>
</tr>
<tr>
<td>7</td>
<td>0.75</td>
<td>Dark Purple</td>
<td>-</td>
<td>-</td>
<td>+ Saponins</td>
</tr>
<tr>
<td>8</td>
<td>0.80</td>
<td>Yellowish Green</td>
<td>-</td>
<td>-</td>
<td>+ Tannins</td>
</tr>
<tr>
<td>9</td>
<td>0.82</td>
<td>Light Purple</td>
<td>-</td>
<td>-</td>
<td>+ Saponins</td>
</tr>
<tr>
<td>10</td>
<td>0.98</td>
<td>Brown</td>
<td>Orange Brown</td>
<td>+ Alkaloids</td>
<td></td>
</tr>
</tbody>
</table>
Based on table 1, it is obtained that on the thin layer chromatography plate, there are 10 separate stain spots with 8 spots, among which are thought to contain active compounds, namely flavonoids, saponins, tannins and alkaloids. The 2 spots include spots 5 and 6 that glow under UV (Ultra Violet) light at a wavelength of 254 nm but are not detected in the appearance of H$_2$SO$_4$ 10% were H$_2$SO$_4$ 10%, Dragendorff reagent, and FeCl$_3$ 1% reagent. Identification of the compounds separated from the film using the Rf value. The Rf value obtained shows differences in the properties of compounds that can be used to identify compounds\textsuperscript{15}. The results of the test for the confirmation of the ethanol extract of Palu local shallot roots through separation of compounds with TLC and the appearance of spots can be seen in table 1 spots, Dragendorff's reagent and FeCl$_3$ 1%. This is because the compounds in spot 5 and 6 do not react with the appearance of the spots provided, so it is difficult to identify the color of the active compound on spot 5 and 6 which correspond to the alkaloid, flavonoid, tannin or saponin compound class. The 8 spots detected under 254 nm UV light and the appearance of the spots are as follows:

**Alkaloids**

Based on Figure 1 on separation by thin layer chromatography using chloroform: methanol (8:2) as eluent, it can be seen on spot 10 with Rf value 0.98 cm; 0.12 cm; 0.18 cm; 0.3; and 0.52 cm appeared blackish green, light green, light green and blackish green after spraying H$_2$SO$_4$ 10% and black after spraying with FeCl$_3$ 1% reagent which was thought to be a flavonoid compound. According to\textsuperscript{17}, the more polar groups you have, the stronger the interaction between the sample and the silica plate will result in the compound having a low Rf value. According to\textsuperscript{18}, detection of flavonoids is carried out by spraying FeCl$_3$ 1% which causes a strong green or black color.

**Saponins**

Based on Figure 1 on the separation by thin layer chromatography using chloroform: methanol (8:2) eluent, it can be seen that on spots 7 and 9 with Rf of 0.75 cm and 0.82 cm, dark purple and light purple colors appear after spraying with H$_2$SO$_4$ 10% which is thought to be a saponin compound. According to\textsuperscript{19}, saponin produces purple and dark purple spots after spraying with H$_2$SO$_4$ 10% in ethanol.

**Tannins**

Based on Figure 1 on the separation by thin layer chromatography (TLC) using chloroform: methanol (8:2) eluent, it can be seen that spot 8 with Rf 0.80 cm appears yellowish green after spraying H$_2$SO$_4$ 10% which is thought to be a tannin compound. According to\textsuperscript{20}, in the based on Figure 2 shows the separation of compounds with TLC using chloroform: methanol (8:2) eluent, it can be seen in spots 10 with an Rf of 0.98 cm which is thought to be an alkaloid compound capable of providing an inhibition zone against Staphylococcus aureus bacteria. According to\textsuperscript{21} that alkaloid compounds have the ability to inhibit bacterial growth/as antibacterial. The mechanism of alkaloids as antibacterials is affirmation test of tannin compounds in the eluent ratio of chloroform: ethanol:water (25:1:0.5), tannin compounds are green under 254 nm UV light and 366 nm UV and yellowish green after sprayed H$_2$SO$_4$ 10%.
Antimicrobial Activity Test with Contact Bioautography Method

The results of KLT-contact bioautography of antimicrobial compounds from ethanol extract of local Palu shallot roots against *Staphylococcus aureus* and *Candida albicans* can be seen in Figures 2 and 3 below:

*Fig. 1: The results of separation of compounds by TLC a) under 254 nm UV light; b) After spraying H$_2$SO$_4$ 10%; c) After spraying Dragendorff’s Reagent and d) After spraying with FeCl$_3$ 1%*

*Fig. 2: Results of Contact Bioautography of Antimicrobial Compounds Against *Staphylococcus aureus***

*Fig. 3: Results of Contact Bioautography of Antimicrobial Compounds Against *Candida albicans***

to disrupt the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not formed completely and causes cell death\textsuperscript{22}. Alkaloid base groups contain nitrogen which reacts with amino acids, resulting in changes in the structure and arrangement of amino acids which disrupt the genetic balance of the DNA chain and encourage bacterial cell lysis\textsuperscript{23&24}.

Based on Figure 3 shows the separation of compounds with TLC using chloroform: methanol (8:2) eluent, it can be seen in spots 10 with an Rf of 0.98 cm which is thought to be an alkaloid compound capable of providing an inhibition zone against the fungus *Candida albicans*. According to\textsuperscript{25} that alkaloid compounds can act as antifungal.

According to\textsuperscript{26} alkaloid compounds work by inhibiting the biosynthesis of fungal nucleic acids, so that fungi cannot develop and
eventually die. The mechanism of alkaloid antifungal activity is by inserting between the cell wall or DNA and then preventing the replication of fungal DNA so that fungal growth will be disrupted\(^\text{27}\).

Meanwhile, according to\(^\text{28}\) the mechanism of alkaloids as antifungal is by binding strongly with ergosterol, causing leakage of the cell membrane, which causes permanent cell damage and fungal cell death. In inhibition growth \textit{Staphylococcus aureus} and \textit{Candida albicans}, the content has different mechanism of action. Flavonoids, alkaloid and tannins works by destroying cell walls microbial and their cytoplasmic membrane so that causes protein denaturation. Flavonoids it works by inhibiting DNA gyrase thus stopping the formation process double stranded DNA in microbial, whereas tannins can inhibit growth microbial protoplasmic so that denaturation of the protein and of will eventual lead to bacterial lysis. The presence of these secondary metabolites will inhibit the growth of \textit{Staphylococcus aureus} and \textit{Candida albicans}. The higher the concentration of active ingredients in the shallot root, the stronger the inhibition of microbial growth\(^\text{20}\).

**Conclusion**

Based on the results of the study, it can be concluded that the antimicrobial compounds that provide inhibitory activity are thought to be alkaloid compounds with an Rf of 0.98 cm against \textit{Staphylococcus aureus} and \textit{Candida albicans}.

**Conflicts Of Interest**

The authors declare no potential conflict of interest.

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تحليل بيولوجي باستخدام كروماتوغرافيا الطبيعة الرقيقة المضادة للميكروبات من مستخلص الأيثانول من جذور كراث بالو المحلي (اليم سيبا اجريجاشن) ضد المكروبات العنقودية الذهبية والمبيضات البيضاء

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كراث بالو المحلي (اليم سيبا اجريجاشن) من المحاصيل المحلية و يمكن أن تنمو جيداً في المناخ الجاف، وخاصة في وادي بالو. يتضمن محتوى المركبات الموجودة في كرات بالو المحلي مركبات الفلافونويد والعنقودية، والصيحيين والفلوريد، عدة فئات من مركبات الفلافونويد والعنقودية، والصيحيين والفلوريد.

تم تحديد مجموعة المركبات النشطة المضادة للميكروبات على لوحة كروماتوغرافيا الطبيعة الرقيقة بسبان الإيثانول لجذور كراث بالو المحلي والتي يمكن أن تثبيط الميكروبات العنقودية الذهبية والمبيضات البيضاء باستخدام تحليل بيولوجي باستخدام كروماتوغرافيا الطبيعة الرقيقة. تم تحديد مجموع المركبات المضادة للميكروبات على لوحة كروماتوغرافيا الطبيعة الرقيقة بسبان الإيثانول لجذور كراث بالو المحلي والتي يمكن أن تثبيط الميكروبات العنقودية الذهبية والمبيضات البيضاء باستخدام كروماتوغرافيا الطبيعة الرقيقة.

وهي الفلافونويد (RF 0.12) والفلوريد (RF 0.56) والثانيين (RF 0.18) والصيحيين (RF 0.67) والفلوريد (RF 0.98). وتم تحديد نسب تثبيط الميكروبات للمكروبات مختبرة كانت مكونات المكروبات من نوع كروماتوغرافيا الطبيعة الرقيقة وRF 0.98 وRF 0.56 وRF 0.18.

وأعتقد أن الباقر التي تثبيط نمو الميكروبات المختبرة كانت مكونات كروماتوغرافيا الطبيعة الرقيقة وRF 0.98 وRF 0.56 وRF 0.18. وRF 0.98 وRF 0.56 وRF 0.18.